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ERYTHROLEUKOSIS AND THE ANEMIAS OF THE FOWL*

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WITH THE ASSISTANCE OF RUTH KLINGELHOFER, B.S., AND CHARLES BREEDIS

PHILADELPHIA

In the course of his studies on the transmissible leukemia of fowls Ellermann [1] observed the frequent occurrence of anemia. On microscopic examination of various organs, particularly of the liver, spleen and bone marrow, many of the anemic birds showed an engorgement of the capillaries with lymphoid cells. On the basis of this characteristic, Ellermann considered the condition, to which he at first gave the name intravascular lymphoid leukosis, a distinct disease. His later studies on the nature of these lymphoid cells led him to surmise that they were progenitors of erythrocytes, and he accordingly changed the name of this pathologic condition to erythroleukosis.

Many of the birds inoculated by Ellermann with the virus of leukemia showed anemia during life, but on microscopic examination of the organs did not reveal the characters of erythroleukosis. These cases are designated by him as simple anemia. Simple anemia and erythroleukosis, according to Ellermann, are varieties of the same disease with all possible transitional forms existing between them.

Most of the investigators working with the leukemia of fowls have observed the occurrence of anemia, but none of them, so far as I am aware, has described cases of erythroleukosis. Data on the spontaneous occurrence of erythroleukosis have been wanting. It is not known whether chronic severe anemia in the bird would resemble erythroleukosis. Cases of erythroleukosis were probably observed by Andersen and Bang [2] and by McGowan,[3] but were not separated from myeloid leukosis.

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* Submitted for publication, Nov. 21, 1930.

* From the Henry Phipps Institute, University of Pennsylvania.

* This investigation has been supported by the Fund for the Study of Leucemia and Related Diseases.

1. Ellermann, V.: The Leucosis of Fowls and Leucemia Problems, London, Gyldenal, 1927; Virchows Arch. f. path. Anat. **228**:247, 1920.

2. Andersen, C. W., and Bang, O.: Festkrift til B Bang, Copenhagen, 1928, p. 353.

3. McGowan, J. P.: Pernicious Anemia, Leucemia and Aplastic Anemia, New York, Paul B. Hoeber, 1927.

Recently Bedson and Knight [4] described in the fowl a severe spontaneous anemia, which is apparently not erythroleukosis and when transmitted to normal fowls is not capable of producing either progressive anemia or erythroleukosis.

Erythroleukosis offers an excellent opportunity for the study of the lymphoid precursors of erythrocytes.

ERYTHROPOIESIS IN THE BIRD

The bone marrow is the only organ of the adult bird in which red cells are formed. The structure and function of the avian marrow, although comparatively simple, are not wholly understood. The marrow is formed by a network of blood vessels, lined with a single layer of endothelium, some dilated, others collapsed. The tissues between the vessels, commonly called trabeculae or parenchyma, contain fat or some homogeneous "gelatinous" material.

Bizzozero and Torre [5] recognized that erythropoiesis and granulocytopoiesis are sharply separated in the marrow of the bird, the former occurring within the venous capillaries, the latter extravascularly in the parenchyma of the marrow. Their observations have been repeatedly confirmed (Van der Stricht,[6] Sabin [7]), and the extensive material presented in this report also substantiates this point of view. Indeed it would seem that when under pathologic conditions erythrogenesis occurs outside of the marrow it is in most, if not in all, instances an intravascular process.

The circulatory system of the marrow is thus intimately connected with the formation of red cells. Sabin,[7] in her recent review on the bone marrow, summarized the available evidence pointing to a closed circulation as recognized by Bizzozero. Attention is called to the significant difference between dilated and collapsed vessels, sluggish flow favoring erythropoiesis (Bizzozero), presumably through low tension of oxygen. Maximow,[8] following Mollier's [9] idea, conceived the erythropoietic sinusoids of the marrow as vessels with fenestrated walls formed by histiocytes with embryonic potencies, capable of constriction and dilation. The former view (Sabin [7]) explains the delivery of the newly formed red blood cells by the opening up of the collapsed capillaries and explains the delivery of the white cells by active migra-

4. Bedson, S. P., and Knight, E.: J. Path. & Bact. **27**:239, 1924.

5. Bizzozero, G.: Arch. f. mikr. Anat. **35**:424, 1890.

6. Van der Stricht, O.: Arch. de biol. **12**:199, 1892.

7. Sabin, F. R.: Physiol. Rev. **8**:191, 1928.

8. Maximow, A.: Bindegewebe und blutbildende Gewebe, in Möllendorff: Handbuch der mikroscopischen Anatomie, Berlin, Julius Springer, 1927, vol. 2.

9. Mollier, S.: Arch. f. mikr. Anat. **76**:608, 1910-1911; **74**:474, 1909.

tion; the latter emphasizes contraction of the histiocytic cells and the fenestrae of the capillaries in explaining the entrance of nonmotile cells into the circulation.

The immediate precursors of erythrocytes are erythroblasts (Bizzozero,[5] Denys [10]). These in the bird are characterized by being round. Their cytoplasm has a peculiar color, given to it by the varying amount of hemoglobin and by the so-called basophil substance that they contain. The more mature forms contain relatively much hemoglobin and are only slightly basophil (erythroblasts II, Sabin [7]). They will be designated in this report as polychrome erythroblasts, a term introduced by Gabritschewski. The younger forms of the erythroblasts contain only traces of hemoglobin and much "basophil substance" (erythroblasts I, Sabin). In this communication they will be called, following the terminology of Ferrata, basophil erythroblasts. The nucleus of the erythroblast has a tendency to form angular clumps of chromatin, giving a characteristic (sometimes called cartwheel) arrangement to the nuclear chromatin. This structure is of particular importance in identifying young erythroblasts.

The origin of the erythroblast is much disputed. Löwit and Denys [10] found that the earliest forms lack hemoglobin and resemble lymphocytes. This view, first denied by Bizzozero, is now generally accepted. The immediate precursor of the erythroblast appears to be mostly a large, round cell with strongly basophil, homogeneous cytoplasm, having a large vesicular nucleus, and resembling closely the younger forms of the erythroblast. The primitiveness of this cell is also indicated by the presence of nucleoli. The name proerythroblast was given to them by Ferrata and de Negreiros-Rinaldi; [11] "megaloblast" (Naegeli) is frequently used in a similar sense. Maximow recognized the existence of proerythroblasts in mammals, but erythroblasts in the bird are shown in his work [8] as derivatives of large lymphoid cells, called by him, with Ferrata, "hemocytoblasts." [12] The main difference between proerythroblasts and their precursors, the hemocytoblasts, is that the cytoplasm of the former is even more basophilic than the cytoplasm of the latter, and that it has a slightly less basophilic perinuclear area. Furthermore, the structure of the nucleus has already some of the characters of the erythroblast. Both possess nucleoli.

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10. Denys, J.: Cellule **4**:200, 1888.

11. Ferrata, A., and de Negreiros-Rinaldi: Virchows Arch. f. path. Anat. **215**:77, 1914.

12. The terms lymphoidocyte (Pappenheim, A.: Folia haemat. **5**:511, 1908) and erythrogony (Helly, K.: Beitr. z. path. Anat. u. z. allg. Path. **49**:15, 1910) refer to similar cells.

Beyond the erythroblastic stage the erythropoiesis of the bird is not definitely known. Aside from the view of Maximow already mentioned, according to which erythroblasts are derived from the assumed multipotent large lymphocytes (hemocytoblasts), the idea supported by Sabin and her co-workers found wide acceptance, namely, that erythroblasts originate from endothelial cells. The origin of erythroblasts from endothelium during early embryonic life seems beyond dispute.[7]

MATERIAL OF STUDY

In the course of our study of the transmissible leukemia of fowls, erythroleukosis or a combination of erythroleukosis and myeloid leukosis developed in about half of the inoculated birds. Moreover, several spontaneous cases of erythroleukosis have been observed. One of these was found in association with sarcoma,[13] and another similar case occurred among the fowls inoculated with leukemic organs. In one bird repeated injections of benzene were followed by the development of erythroleukosis, and in another partial destruction of the bone marrow was followed by a similar event. Erythroleukosis was found in one chicken exposed to the ectoparasites of a leukemic fowl. These and a few atypical cases, most of them closely followed from the onset of illness until death, are the subject of this paper. In addition, a few experiments will be described that were performed to produce severe anemia by repeated bleeding or by continued administration of blood poisons, and the difference between these types of secondary anemia and erythroleukosis will be discussed.

TECHNIC

Blood Smears.—Blood smears were taken from all birds that appeared pale or ill, and at irregular intervals (from one to three months) from the entire flock.

Blood Counts.—Blood counts were made by diluting blood 1:100 or 1:200 with Toisson solution and counting white and red cells in the same chamber. The counting of normal blood and of pathologic blood other than erythroleukotic is satisfactory by this method. In erythroleukosis there is a continuous transition between red cells and their lymphoid precursors, making the counting of white cells arbitrary and somewhat inaccurate, the lymphoid erythroblasts being thus counted with the white cells. A more accurate method in these cases consists of determining the total number of cells and calculating the number of the various constituents on the basis of the differential counts. With some experience, however, direct counting, a much simpler procedure, gives approximately accurate results.

Recently several investigators described new methods of counting the blood cells of fowls. One of them[14] said that when suspended in Toisson solution the

13. Dr. E. L. Stubbs gave me permission to describe this case.

14. Shaw, A. F. B.: J. Path. & Bact. **33**:833, 1930.

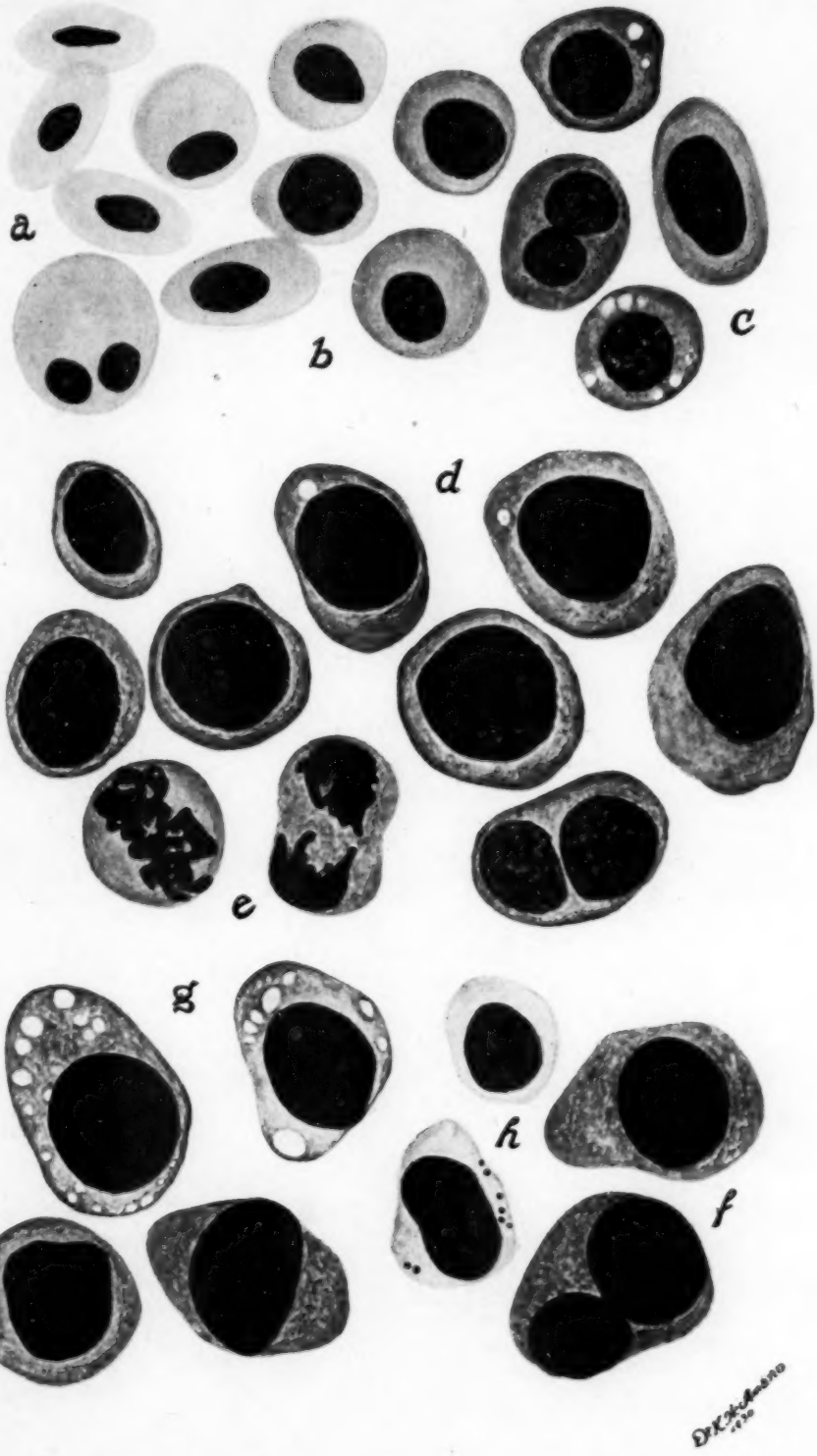

Fig. 1.—The blood cells in erythroleukosis of the fowl; *a*, erythrocytes; *b*, polychrome erythrocytes; *c*, polychrome erythroblasts; *d*, lymphoid erythroblasts; *e*, mitotic figures; *f*, lymphoid cells; *g*, lymphoid cells with vacuoles, and *h*, small lymphocytes.

UNIV. OF ILLINOIS

lymphocytes of the fowl cannot be differentiated from the thrombocytes, but in my experience the differences between them have been marked. The thrombocytes are spindle-shaped not unlike erythrocytes, but they are somewhat smaller, lack hemoglobin and appear very pale in the counting chamber. The lymphocytes are much smaller and round, and take up crystal violet intensely. The thrombocytes were left entirely out of consideration in this study.

Smears from Organs.—Pieces of organs were cut in chicken serum and the larger particles allowed to settle. A smear was made with a small drop of the turbid suspension of cells, as is done with blood droplets. This method served the purpose of comparing the cells present in the blood-forming organs with those of the blood, and also permitted a rapid tentative diagnosis of some tumors.

Other Procedures.—For staining of slides, fixation of organs, staining of sections and nomenclature used, see an earlier publication by Dr. Furth.[15]

ERYTHROLEUKOSIS PRODUCED BY TRANSMISSION

Table 1 is a survey of the cases of erythroleukosis occurring among the fowls inoculated with leukemic material in the course of the experiments in transmission of this disease already described.[15] The number of the bird and that of the series are given to facilitate a correlation of the data presented.

Symptomatology.—The first signs of illness were discovered from 13 to 124 days after inoculation, with an average of 47 days. In most instances slight paleness attracted attention, while the bird still appeared active and the breast muscle was fairly strong. In a few instances, however, the comb was red, and only the routine examination of the blood led to the discovery of the pathologic condition. These cases are of particular interest because they indicate that erythroleukosis does not arise from preceding anemia. That the onset of spontaneous erythroleukosis may be similar is shown in case 366 (p. 22).

Many of the fowls died shortly after the disease became manifest while apparently in good physical condition, but others lost weight and became extremely emaciated. The duration of the illness averaged 19 days, and the birds lived from 21 to 204 days after inoculation. In several instances death was caused by an intercurrent disease, such as purulent infection of the upper respiratory tract or of the air sacs. Recovery was observed in two cases, in which the diagnosis was based solely on examinations of the blood.

Blood Picture.—The principles followed in differentiating immature erythrocytes have been described. Figures 1 and 2 illustrate the various types of cells of the erythroblastic series. A peculiar large lymphoid cell (figs. 1 *f* and *g* and 2 *E*) has been included, because it has not been seen in conditions other than erythroleukosis. The nucleus is large and vesicular and appears to be homogeneous. Nucleoli

15. Furth, J.: J. Exper. Med. **53**:243, 1931.

TABLE 1.—*Erythroleukosis and Anemia Among Fowls Inoculated with Leukemic Material*

Chicken	Series	Weight, Gm., at Time of Inoculation	Weight, Gm., at Time of Death	Material Used for Transfer	Period of Incubation, Days	Duration of Illness, Days	Length of Life after Inoculation, Days	Summary of the Examination of the Blood *
177	I	920	1,150	Organ emulsion 198	79	2	81	Erythroleukosis (erythroblastic), incomplete osteosclerosis
178	IV-J	1,310	850	Blood 365; myeloid leukosis	69	16	85	Erythroleukosis (atypical)
269	IV-J	1,750	1,450	Blood 365, myeloid leukosis	69	13	82	Erythroleukosis (atypical)
270	II-A	920	1,110	Blood 177, erythroleukosis	43	24	67	Erythroleukosis (erythroblastic)
276	II-A	850	1,280	Blood 177, erythroleukosis	132	72	204	Erythroleukosis (erythroblastic and lymphoid), with osteosclerosis
328	III-A	660	.....	Organ 266, myeloid leukosis	69	1	70	Erythroleukosis (erythroblastic)
358	III-B	940	.....	Organ 266, myeloid leukosis	...	..	169	Erythroleukosis (erythroblastic), with mild myeloid leukosis
455	IV-B	1,040	.....	Blood 328, erythroleukosis	114	19	133	Erythroleukosis (erythroblastic), mild
466	IV-D	1,100	1,775	Plasma 361, myeloid leukosis	71	16	87	Anemia secondary to myeloma
489	IV-C	980	1,230	Blood 361, myeloid leukosis	90	9	99	Erythroleukosis (mainly erythroblastic)
499	IV-C	760	1,170	Berkefeld filtrate 361, myeloid leukosis	100	91	191	Erythroleukosis (lymphoid, changing to erythroblastic)
511	IV-J	910	660	Blood 365, myeloid leukosis	46	14	60	Erythroleukosis (erythroblastic), with myeloid leukosis
553	V-B	730	.....	Organ emulsion 399, lymphoid leukosis	117	29	146	Erythroleukosis (lymphoid), recovered
565	V-C	935	820	Bone marrow 505, myeloid leukosis	131	21	154	Erythroleukosis (erythroblastic), with myeloid leukosis
575	IV-G	1,030	.....	Blood 365, myeloid leukosis	64	17	60	Erythroleukosis (lymphoid), with mild myeloid leukosis
599	III-C	870	.....	Blood 276, erythroleukosis	20	11	31	Erythroleukosis (lymphoid)
602	III-C	690	640	Blood 276, erythroleukosis	28	10	38	Myeloid leukosis, with erythroleukosis (lymphoid)
604	III-C	800	650	Blood 276, erythroleukosis	26	4	30	Erythroleukosis (lymphoid), with myeloid leukosis
605	III-C	800	820	Blood 276, erythroleukosis	26	3	29	Erythroleukosis (lymphoid)
612	IV-H	750	.....	Blood 276, erythroleukosis	44	4	48	Erythroleukosis (erythroblastic)
614	IV-H	700	.....	Blood 276, erythroleukosis	28	14	42	Myeloid leukosis, with erythroleukosis (erythroblastic)
615	IV-H	740	.....	Blood 365, myeloid leukosis	56	39	95	Erythroleukosis, mild
627	IV-H	840	.....	Frozen and thawed blood 365, myeloid leukosis	44	2	46	Erythroleukosis (lymphoid)
647	V-C	700	730	Blood 490, myeloid leukosis after treatment with distilled water	124	26	150	Erythroleukosis (erythroblastic), with myeloid leukosis
657	IV-J	740	660	Blood 365, myeloid leukosis	16	17	33	Erythroleukosis (erythroblastic)
653	IV-J	840	900	Blood 365, myeloid leukosis	32	1	33	Erythroleukosis (lymphoid)
655	IV-J	700	555	Blood 365, myeloid leukosis	43	23	67	Erythroleukosis (erythroblastic), with myeloid leukosis
656	IV-J	700	975	Plasma 365, myeloid leukosis	35	5	40	Erythroleukosis (lymphoid)
661	IV-J	930	940	Plasma 365, myeloid leukosis	35	6	41	Erythroleukosis (lymphoid)
670	IV-J	900	.....	Frozen and thawed blood 365, myeloid leukosis	25	4	29	Erythroleukosis, mild
675	IV-J	845	1,070	Blood 365 after treatment with distilled water	113	7	120	Erythroleukosis (erythroblastic), with sarcoma
681	IV-J	790	800	Blood 365 after treatment with distilled water	43	12	55	Erythroleukosis, with myeloid leukosis
691	IV-L	920	.....	Blood 365, myeloid leukosis	20	1	21	Erythroleukosis, incipient
694	IV-L	680	700	Blood 365, myeloid leukosis	16	9	25	Erythroleukosis (erythroblastic)
698	IV-M	720	1,000	Blood 604, myeloid leukosis	29	20	49	Erythroleukosis (erythroblastic)
705	IV-M	1,580	1,140	Plasma 604, myeloid leukosis	55	9	64	Erythroleukosis (erythroblastic), with myeloid leukosis
715	IV-N	770	.....	Blood 604, myeloid leukosis	27	23	..	Erythroleukosis, recovered
723	IV-N	800	640	Plasma 604, myeloid leukosis	24	31	55	Erythroleukosis (erythroblastic)
739	VI-A	900	900	Blood 572, myeloma	54	26	80	Erythroleukosis (erythroblastic), with myeloid leukosis
757	V-F	770	.....	Blood 499, erythroleukosis	23	111	134	Erythroleukosis (erythroblastic)
759	V-F	840	750	Blood 499, erythroleukosis	22	32	54	Erythroleukosis (erythroblastic)
767	V-F	590	550	Plasma 499, erythroleukosis	28	27	55	Erythroleukosis (erythroblastic)
795	IV-O	850	750	Blood 600, erythroleukosis, myeloid leukosis	13	9	22	Erythroleukosis (lymphoid)
798	VI-B	830	.....	Blood 643, myeloid leukosis	67	1	68	Erythroleukosis (lymphoid)

* The type of erythroleukosis (lymphoid or erythroblastic) refers to the dominating cell in the blood stream.

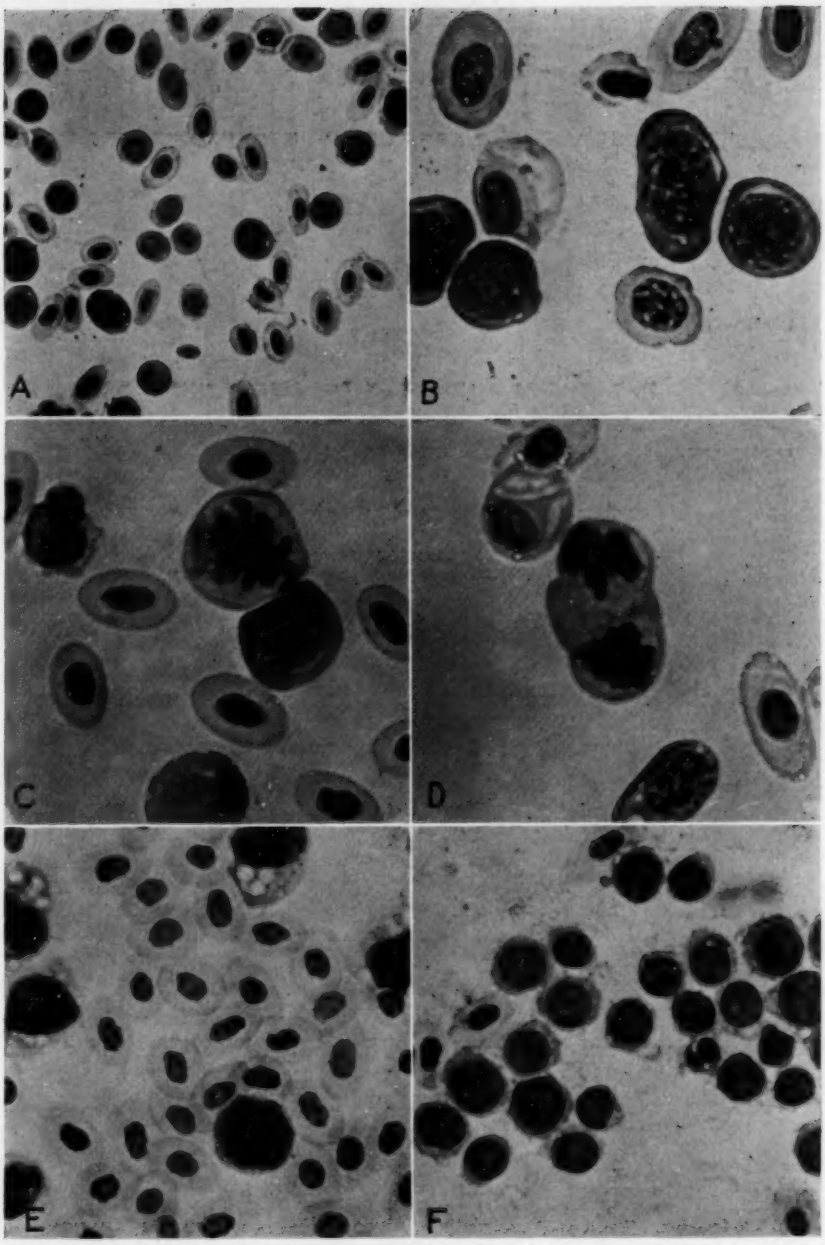

Fig. 2.—*A*, a blood smear in erythroleukosis of the erythroblastic type; *B*, basophil erythroblasts shown with higher magnification; about × 1,000; *C*, one mitotic figure, one basophil erythroblast, one lymphocyte and one polymorphonuclear leukocyte with rod-shaped granules; *D*, mitotic figure; *E*, blood smear in erythroleukosis of the lymphoid cell type, showing one lymphoid erythroblast and five lymphoid cells; *F*, smear from the liver in erythroleukosis, showing the erythroblastic character of the lymphoid cells of the capillaries of the liver.

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TABLE 2.—*Blood Picture in Erythroleukosis Produced by Transmission*

Chicken	Date of Examination	White Blood Cell Count in 1000's	Red blood Cell Count in 1000's	Hemoglobin (Sahli)	Polychrome Erythrocytes#	Polychrome Erythroblasts	Basophil Erythroblasts	Proerythroblasts	Primitive Lymphoid Cells†	Same with Vacuoles	Mitotic Figures	Large Mononuclears‡	Promyelocytes¶	Myelocytes	Metamyelocytes	Polymorphonuclears	Mast Cells	Lymphocytes†
177	4/16	Normal	.....	..	Few	.....	..	..	..	..	..	12	..	..	..	25	1	62
3/26-6/14*	6/12	57	445	15	Few	Abundant	23	4	3	1	..	23	1	5	..	30	5	5
178	2/25	Normal	.....	..	.....	.....	..	..	..	..	..	14	..	..	..	50	..	36
1/16-4/11	3/26	15	1,360	32	Many	Few	6	2	5	1	..	25	..	..	..	11	4	46
	4/ 3	12.5	830	19	Many	Few	5	5	12	13	..	11	..	..	..	7	..	47
	4/ 7	Low normal	.....	..	Abundant	Few	..	2	16	12	..	2	..	..	..	8	..	60
269	6/24/29	High normal	.....	..	.....	.....	..	..	1	..	..	15	..	..	..	73	1	10
1/16-4/8	2/27/30	Normal	.....	..	Few	Few	3	..	..	..	..	6	..	..	..	25	6	60
	3/26	19	690	12	Many	Few	5	4	4	3	..	11	1	..	..	19	6	47
	4/ 4	45	410	8	Many	Many	14	..	13	2	..	15	4	1	..	45	..	6
	4/ 8	38	287	9	Many	Few	2	..	12	1	1	16	3	..	..	54	3	8
276	7/27	Normal	.....	..	.....	.....	..	..	..	..	..	5	..	..	..	26	10	59
6/14-1/4	11/26	20	2,095	46	Abundant	Many	16	1	2	1	..	15	1	..	..	2	1	61
	1/ 4	81	355	13	Few	Abundant	34	13	10	33	1	2	..	..	..	1	..	6
328	7/26	Normal	.....	..	.....	.....	..	..	..	..	..	19	..	..	..	36	4	41
7/26-1/4	8/ 9	41	2,060	..	.....	.....	..	..	..	..	..	25	..	..	..	37	2	36
	10/ 3	14	1,280	30	Few	Abundant	42	10	15	7	..	4	..	..	1	15	..	6
358	12/ 7	Normal	.....	..	.....	.....	..	..	..	..	..	16	..	..	..	31	6	47
8/29-2/14	2/13	High normal	.....	..	Many	Abundant	30	3	3	6	..	21	7	2	2	5	..	21
499	10/18	Normal	.....	..	.....	.....	..	..	..	..	..	8	..	..	..	43	1	48
10/18-4/18	1/28	48	2,290	..	Few	Few	5	1	16	41	..	3	..	..	..	2	2	30
	1/31	57	1,015	30	Few	Few	5	4	35	30	..	1	..	..	..	1	..	24
	2/ 6	214	1,128	..	Many	Many	25	12	19	13	2	2	..	..	..	2	..	25
	2/26	88	570	14	Few	Abundant	11	11	34	23	..	1	1	..	1	2	1	15
	3/19	13	2,835	49	Few	Few	2	..	..	..	..	6	..	..	..	14	5	73
553	2/28	Normal	.....	..	Very few	.....	..	..	..	..	..	12	..	..	..	33	5	50
12/10-7/7	4/ 8	36	1,250	29	Very few	Very few	1	1	22	1	..	9	2	..	..	1	3	60
	4/20	68	1,160	..	Many	Many	10	1	..	..	..	3	1	..	..	16	6	63
	4/26	Normal	.....	..	Many	Very few	..	..	..	..	..	8	..	..	..	14	4	74
	6/ 2	Normal	.....	..	Many	Few	3	1	8	..	..	6	..	2	..	21	5	54
	6/31	Normal	.....	..	Few	Few	5	..	1	..	..	16	1	1	..	28	9	39
575	2/11	High normal	.....	..	.....	.....	..	..	..	..	..	21	..	..	..	29	..	50
2/26-3/17	2/28	High normal	.....	..	Few	Few	5	..	1	..	..	14	..	..	..	22	6	52
	3/ 3	17	2,145	41	Very few	Few	6	2	1	..	1	12	..	..	..	9	3	66
	3/13	26	1,005	20	Few	Few	5	..	21	2	2	14	3	..	..	..	5	48
	3/17	95	770	..	Many	Many	11	8	20	3	2	17	..	6	..	14	1	18
602	1/ 3	Normal	.....	..	.....	.....	..	..	..	..	..	5	..	..	..	19	5	71
1/4-2/11	2/ 5	Normal	.....	..	Few	Many	16	1	5	..	1	7	..	..	..	5	1	64
	2/10	55	1,390	23	Very few	Very few	..	..	9	25	..	49	1	2	3	4	..	7

655	1/16	Normal	.....	..	.....	.....	..	..	..	..	..	18	..	..	..	27	2	53
1/16–3/24	2/28	High normal	.....	..	Very few	Very few	23	5	22	6	..	2	..		..	..	..	42
	3/11	Mild leukemia	.....	..	Many	Many	20	6	40	10	1	13	..	..	..	1	..	9
	3/22	Low normal	.....	..	Few	Few	8	..	16	4	..	8	..	..	1	3	..	60
656	1/16	Normal	.....	..	.....	.....	..	..	..	..	..	15	..	.	..	44	2	39
1/16–2/25	2/20	High normal	.....	..	Very few	Few	6	3	8	5	..	1	..	..	2	16	1	58
	2/25	182	745	16	Few	Many	7	5	25	18	..	34	2	1	2	..	..	8
675	1/26	High normal	.....	..	.....	.....	..	..	14	..	..	6	..	..	..	45	4	31
1/16–5/6	4/26	Normal	.....	..	Very few	.....	..	..	..	..	..	22	..	..	..	48	..	30
	5/ 2	Normal	.....	..	Many	Few	1	..	..	..	..	10	..	..	1	45	1	42
	5/ 5	Normal	.....	..	Few	Few	2	..	10	..	.	6	..	..	..	34	2	46
681	1/16	Normal	.....	..	.....	.....	..	..	..	..	..	10	..	1	2	15	4	68
1/16–3/12	3/ 3	137	2,650	42	Very few	Few	2	1	18	54	1	4	..	..	2	2	1	15
	3/11	315	1,040	23	Few	Many	28	41	..	..	5	21	3	..	..	..	..	2
696	1/30	Normal	.....	..	.....	.....	..	..	..	..	..	7	..	..	..	51	4	38
1/13–3/20	3/ 3	30	2,540	57	Many	Many	2	..	6	..	..	8	1	..	..	15	2	66
	3/18	340	2,580	44	Few	.....	..	..	..	..	..	37	51	4	2	2	..	4
715	2/ 4	Normal	.....	..	.....	.....	..	..	..	..	..	8	..	..	..	28	7	57
2/4–7/28	2/28	Normal	.....	..	Many	Many	13	1	4	1	..	3	..	..	..	19	2	57
	3/ 3	22	2,505	48	Very few	Very few	8	1	4	1	..	15	..	.	..	6	3	62
	3/28	Normal	.....	..	.....	.....	..	..	..	..	..	21	..	..	..	23	3	53
	4/26	Normal	.....	..	.....	.....	..	..	..	..	..	9	..	..	..	26	7	58
723	2/ 4	Low normal	.....	..	.....	.....	..	..	..	..	..	12	..	..	..	20	..	68
2/4–3/31	3/18	Normal	.....	..	Few	Few	11	3	6	2	..	17	..	..	..	6	1	54
	3/20	37	1,150	28	Few	Many	14	4	10	4	..	24	..	..	..	1	2	41
	3/29	High normal?	.....	..	Many	Abundant	32	3	7	1	1	33	1	6	2	3	..	11
757	2/ 6	Normal	.....	..	.....	.....	..	..	..	..	..	20	..	..	..	36	6	38
2/6–6/20	3/20	40	2,850	66	Very few	Very few	8	3	14	4	1	21	..	..	1	9	..	39
	3/26	58	2,530	50	Very few	Very few	8	1	26	3	1	11	1	..	1	9	2	37
	6/16	Mild leukemia	.....	..	Very few	Very few	16	4	8	..	2	61	1	..	..	..	..	8
759	2/ 6	Normal	.....	..	.....	.....	..	..	..	..	..	18	..	..	..	50	4	28
2/6–4/1	2/28	Mild leukemia	.....	..	Few	Few	6	..	19	48	1	3	2	1	..	15	..	5
	3/ 3	36	2,000	50	Few	Few	12	3	8	1	2	19	2	8	..	10	3	32
	3/26	Normal	.....	..	Few	Few	13	1	20	3	..	10	..	3	..	10	3	37
767	2/ 7	High normal	.....	..	.....	.....	..	..	..	..	..	26	..	..	..	46	..	28
2/16–4/24	3/18	33	2,150	45	Very few	Very few	1	..	..	..	..	27	..	..	..	14	..	58
	4/12	85	620	..	Few	Abundant	61	8	..	11	3	7	..	1	..	2	2	5
798	2/27	Normal	.....	..	.....	.....	..	..	..	..	..	15	..	..	..	52	7	26
2/27–5/6	4/14	Normal	.....	..	.....	.....	..	..	..	..	..	2	..	..	..	15	..	83
	5/ 6	42	770	15	Abundant	Abundant	35	7	15	6	2	3	..	3	..	19	1	9

* The dates given with the number of the fowl show the interval between inoculation and death. The first number of the date indicates the month, the second the day of the month.

For the principles followed in differentiating atypical cells, see pages 3 and 5.

† Lymphocytes of small and medium size were counted together. The large lymphocytes were counted with the primitive lymphoid cells.

‡ These figures include primitive cells resembling large mononuclear leukocytes and the "poikilonuclear" cells of Ellermann. An analysis of these cells will be attempted in a report on myeloid leukosis.

¶ Large mononuclear leukocytes with purple granules; these cells are characteristic of myeloid leukosis of fowls.

are present. The cytoplasm is narrow and very basophil, appears round and often has small lingulate processes. The extreme basophilia differentiates these cells from hemocytoblasts, and lack of the characteristic nuclear structure, from proerythroblasts. In a large percentage vacuoles are seen. In fixed and stained sections differentiation is difficult, if not impossible.[16] These cells are here given temporarily the designation "lymphoid" cells, and they are tabulated as immediate precursors of proerythroblasts, although this genetic reconstruction based on morphologic grounds requires further evidence. It would seem possible that many of these cells are lymphoid erythroblasts that have undergone degeneration, hence the structureless nucleus and vacuolization of the cytoplasm. Direct smear from the infiltrated organs would seem to support this view (p. 14).

The blood cells of healthy fowls will not be reviewed here. The large mononuclear cells seen in myeloid leukemia will be called, with Ellermann, "large mononuclear cells (myeloblasts)" and similar cells with purple granules "promyelocytes." They will be described more fully in a paper on myeloid leukosis. Some uncertainty as to the correctness of this nomenclature should be kept in mind.

At the onset of the disease, in contrast with secondary anemia, there appears to be an adequate number of erythrocytes in the circulation, and the amount of hemoglobin is likewise not considerably diminished; e. g., in fowl 757 there were 2,850,000 red blood cells at the onset of disease; the red blood cells in fowl 750 numbered 2,000,000 even before death. Polychromatophil erythrocytes are present in moderate amounts, whereas erythroblasts, among them the early forms, are relatively numerous. In some instances lymphoid cells appear in the circulation in considerable numbers, while anemia is extremely slight (e. g., fowl 681).

The blood picture during the course of illness is given in table 2. Since there is a continuous series between erythrocytes and their lymphoid precursors, the differential counts given naturally are arbitrary and somewhat inaccurate.

The medium or large lymphoid cells are counted with the white cells, and if they appear in considerable numbers the blood picture becomes leukemic. This study includes eleven such cases with fatal outcome, which in table 1 are designated as "erythroleukosis (lymphoid)" to distinguish this from the more common erythroblastic form. In seven of these cases the figures for white cells were: 42,000 (798), 67,000 (605), 95,000 (575), 101,000 (661), 164,000 (579), 182,000

16. Maximow (footnote 8, fig. 79).

(656) and 201,000 (602). The dominating cell was the lymphoid cell described. Thus erythroleukosis of the lymphoid cell type may be distinctly leukemic.

When these cases are considered separately, it becomes evident that the period of incubation (thirty-six days) is below the average, and that their course is more fulminating, the average length of illness being seven days. This type may therefore be understood as a more acute form of erythroleukosis.

It is obvious from table 2 that a sharp line does not exist between erythroleukosis of the lymphoid cell type and erythroleukosis of the erythroblastic type. Indeed the continuous transitional forms between the two support the assumption that they are varieties of the same process. A change of type from erythroblastic to lymphoid (e. g., 575 and 602) or the reverse (499) may likewise occur. In microscopic anatomy of organs no marked difference is observed between these pathologic conditions.

Ellermann's statements that the leukocytes are outside the essential change of erythroleukosis, and that, if the "lymphoidocytes" are not considered, one gets a normal percentage of each of the various types of white cells, are not supported by the data presented in table 2. A considerable decrease of the polymorphonuclear leukocytes appears to be the rule, and agranulocytosis is not uncommon. The behavior of the lymphocytes is less constant, but their number likewise decreases considerably in almost every pronounced case.

The presence of myelocytes is not uncommon in erythroleukosis. It is seen from table 2 that myelocytes were not found in smears taken before inoculations, but when blood smears were taken repeatedly after inoculation an occasional myelocyte was seen in almost two thirds of the cases of erythroleukosis, usually at a more advanced stage. Occasionally the number of myelocytes and large mononuclear cells (myeloblasts, Ellermann) became considerable. In most of these cases the sections of the blood-forming organs likewise revealed an involvement of both erythropoietic and granulopoietic systems. Indeed in several instances the microscopic picture of the bone marrow revealed a myeloid involvement not suspected after the examination of blood smears. In a few instances the blood picture at an early stage was that of erythroleukosis, which then changed completely to myeloid leukemia (e. g., 696).

Erythroblasts with double nuclei were described by Ellermann and were considered by him as pathognomonic for erythrocytes and erythroblasts. In figure 1 such cells are reproduced. It is noteworthy that cells with double nuclei were particularly numerous among the primitive lymphoid cells described, pointing to a connection with cells of erythro-

blastic potencies. Occasionally, however, double nuclei were found in cells other than those of the erythroblastic series.

Maximow [17] considered the erythroblast with a double nuclei a degenerated cell. In the microscopic aspect of fixed and stained smears there is no support for this opinion, for this cell appears to differ from the normal erythroblast merely by the double nuclei. It is more probably the result of an atypical division of a cell.[18]

Characteristic inclusions are found not infrequently in large mononuclear cells of myeloid leukemia, and will be described in a subsequent paper. They are rare in erythroleukosis.

Mitotic figures are found in practically every severe case of the transmissible leukosis. In some they are abundant. Figures 1 *e* and 2 *C* and *D* show some of the types seen in erythroleukosis. The large number of mitotic forms in the peripheral blood and in the capillaries of the organs offers the most direct support for the possibility, thus far surprisingly disregarded, that transfer of leukemia of fowls, not unlike transmission of cancer and leukemia of mammals, is bound to tumor cells.

Anatomic Changes.—Bone Marrow: As in myeloid leukemia the bone marrow was involved in all cases of erythroleukosis observed (cf. Schmeisser [19]). However, whereas myeloid leukemia seems to be preceded by an aleukemic stage with maximum myelocytic and myeloblastic hyperplasia of the marrow, the involvement of the peripheral blood in erythroleukosis appears to take place at a very early stage. The disease was diagnosed or suspected before death by reason of the erythroblasts present in the circulation in all cases, with the possible exception of one (503), which will be fully described in later paragraphs. The early involvement of the peripheral blood is probably due to the intravascular nature of erythrogenesis, whereas myeloid leukemia begins in the trabeculae of the marrow. The pathologic changes in erythroleukosis were most marked in the marrow, and in a few cases (178, 269 and 358) the specific involvement of the other organs was very mild, suggesting that the bone marrow is the primary site of erythroleukosis as it is of myeloid leukemia.

The gross appearance of the marrow is characteristic, though it was neither recognized with certainty in all cases nor always differentiated from the marrow of myeloid leukemia. The marrow shows loss or absence of fat. It is reddish or pale grayish, and its consistency is increased, so that it can be removed as a semisolid cylindric mass.

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17. Maximow (footnote 8, p. 388).
18. McGowan (footnote 3, p. 71).
19. Schmeisser, H. C.: Johns Hopkins Hosp. Rep. **17**:551, 1916.

In sections the latent and patent blood vessels can be well made out in the early or in the mild cases. The former vessels show sinusoid dilations and are filled with erythroblasts and lymphoid cells, the latter contain chiefly mature erythrocytes and immature cells such as are seen in the peripheral circulation. The structure of the marrow is preserved (fig. 3 *C*). In the trabeculae, foci of small cells resembling

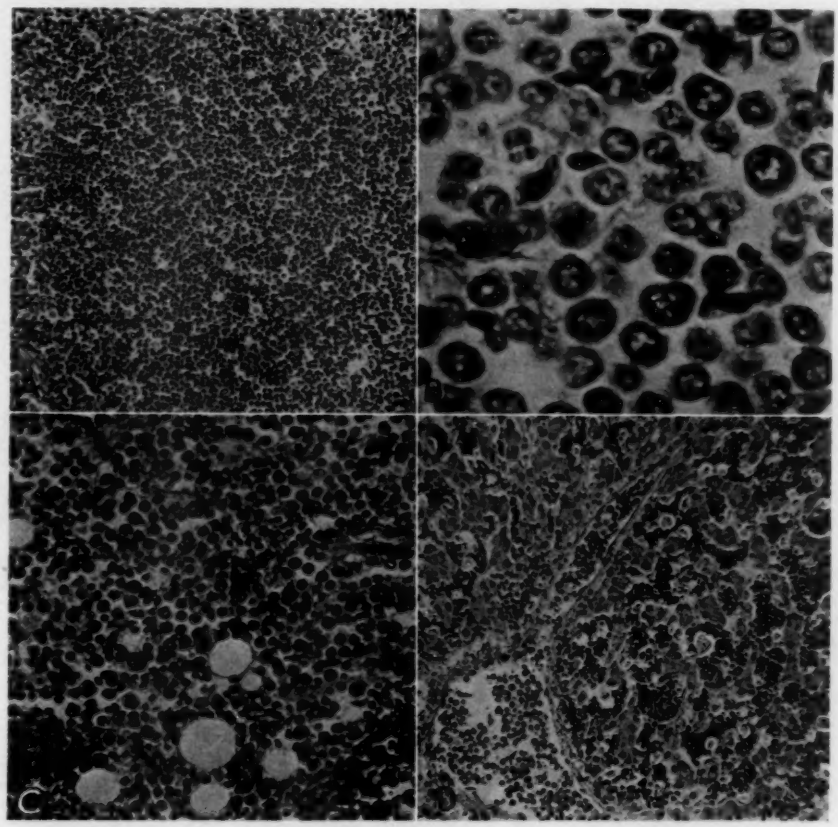

Fig. 3.—*A* and *B*, the bone marrow in advanced erythroleukosis of the fowl; *C*, the bone marrow in incipient erythroleukosis; *D*, the liver in erythroleukosis.

lymphocytes and foci of myelocytes are seen, the latter sometimes somewhat in excess of normal. Figures that could be interpreted as proliferating endothelial cells have not been identified with certainty. As the disease progresses, the crowding of the sinusoids becomes more pronounced, the fat content of the marrow becomes reduced, the trabeculae become narrower, and in an advanced stage the marrow has the appearance of a dense mass of lymphoid cells (fig. 3 *A* and *B*).

These observations are based on examination of the marrow of the femur and tibia. In the air-containing humerus, which was not systematically examined, no change was observed.

Liver: The liver is usually enlarged, but not to the same extent as in lymphoid leukosis. The average weight of the liver of the normal fowl is 2.8 per cent of the weight of the body (Pickens [20]). In twenty-nine cases of erythroleukosis the weight of the birds post mortem varied between 550 and 1,450 Gm.; the average weight was 863 Gm. The weight of the liver varied between 29 and 184 Gm., with an average of 64.5 Gm. (7.5 per cent of the weight of the body). In individual fowls, however, including all mild and some pronounced cases, the liver appeared to be of normal size (178, 269, 358, 503, 511, 575, 602, 605, 612, 613 and 691).

The color of the liver is in most instances distinctly grayish; in a very few instances it is slightly yellowish and fatty. Its consistency is as a rule slightly increased, but occasionally it is very brittle. The surface and cut surface either is homogeneous or shows a fine mottling caused by minute grayish areas.

Thus on gross examination of the liver erythroleukosis can in some instances not even be suspected. The microscopic appearances, however, are characteristic and pronounced in all but a few atypical or mild cases (178, 269, 358, 455 and 691). This specific involvement consists of a selective accumulation of lymphoid cells in the capillaries to a varying degree (fig. 3 *D*). Their location is intravascular, with the exception of an occasional tumor-like accumulation of lymphoid cells. In view of the fact that hemorrhages are found regularly in erythroleukosis, one might assume that such growth is perhaps due to lesions in the endothelial linings of the blood vessels. Associated small foci of myelocytes and myeloblasts, chiefly perivascular, are not uncommon. The character of the myeloid tissue in the livers of fowls will be discussed more fully in a subsequent paper on myeloid leukosis.

To ascertain the character of the lymphoid cells accumulating in the capillaries of the liver, smears were prepared from emulsions of the liver in chicken serum. The cells in such smears resemble lymphocytes very closely, varying greatly in size from that of the small to that of the large lymphocyte. The cytoplasm surrounds the nucleus in the form of a rather regular, narrow, basophil ring, with a clear halo around the nuclear membrane. The nucleus has a distinct network of chromatin, caused by a nodular or linear concentration of the chromatin; the characteristic sharply angular network of the erythroblasts is wanting. Nucleoli are distinct in many of these cells. They are surrounded by a denser mass of chromatin and are, if pronounced, slightly bluish (fig. 2 *F*).

20. Pickens, E. M.: Rep. New York State Vet. Coll., 1917, p. 226.

Although such cells, notably those of smaller size, resemble lymphocytes very closely, the majority of the well preserved cells can be distinctly recognized as lymphoid erythroblasts. The term erythrogony (Helly [21]) would seem to be well chosen for such cells, which appear to be different from large lymphocytes (lymphoblasts).

Spleen: Enlargement of the spleen occurring in most instances to an extent not observed in any other condition known, with moderate

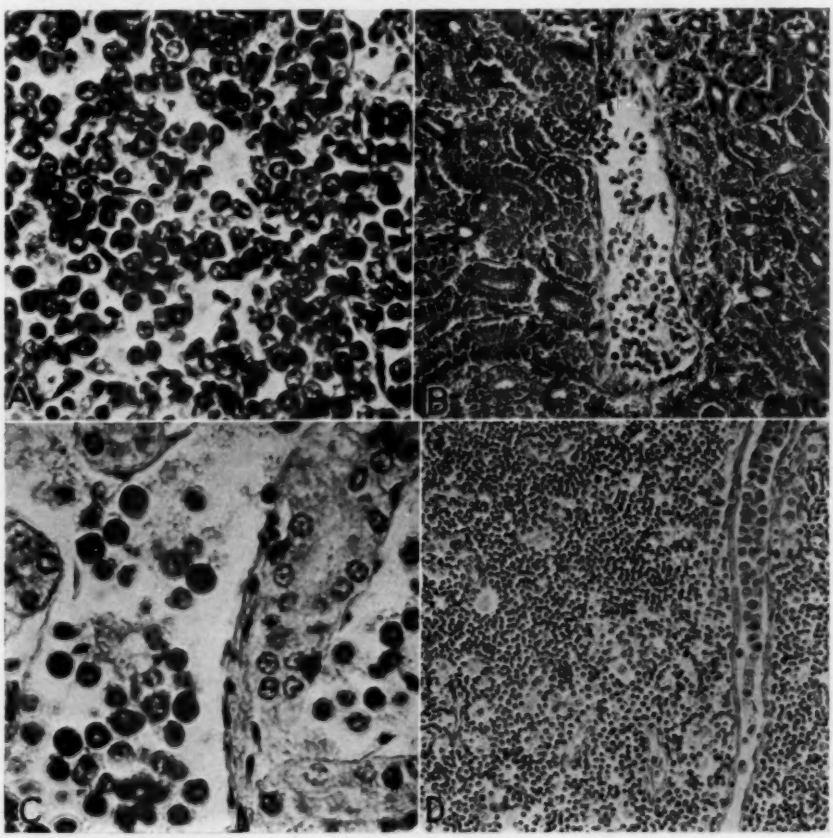

Fig. 4.—*A*, the spleen in erythroleukosis; *B* and *C*, the kidney in erythroleukosis; *D*, the difference in size and in character of the lymphoid cells seen in erythroleukosis and the lymphocytes of the thymus.

enlargement of the liver, appears to be the most striking feature of the gross postmortem examination. The size of the normal spleen is about 0.25 per cent of the weight of the body (Pickens [20]). In twenty-nine cases of erythroleukosis with an average weight of body of 863

21. Helly (footnote 12, second reference).

Gm., the weight of the spleen varied between 0.5 and 21 Gm., with an average of 6.1 Gm. (0.71 per cent of the weight of the body). This relatively low average is due to the fact that in some mild cases the spleen was only slightly enlarged and to the fact that in the atypical cases (178 and 269) it was below normal (0.05 per cent and 0.06 per cent of the weight of the body).

The color of the spleen is grayish brown, uniformly or with a fine mottling of minute pale grayish areas. It is moderately firm.

The involvement of this organ seems to begin by the accumulation of lymphoid cells in the pulp until by progressive accumulation they encroach on the follicles (fig. 4). This process is comparable to the compression of the granulocyte-containing trabeculae of the marrow by the proliferating lymphoid cells in the sinusoids (Ellermann [1]).

Spontaneous rupture of the enormous spleen was the immediate cause of the death of fowl 698.

Thus stasis of the lymphoid cells in capillaries is the dominant feature of erythroleukosis. This, although most constant in the blood-forming organs, is by no means limited to them.

Other Organs: Of the other organs, the kidney (fig. 4 *B* and *C*) and adrenal gland are frequently affected to a moderate degree. The involvement of the lung and heart is likewise common, but less extensive. In mild cases the stasis is localized to parts of the organs, depending apparently on the sluggishness of the flow of blood in these areas. Less frequent and less extensive is the involvement of the thymus (fig. 4 *D*) and thyroid glands, and distinct stasis in the pancreas has been observed in only one severe case (661).

Among the organs rarely affected, the nervous system is of particular importance because of its frequent involvement in paralysis and lymphoid leukosis of the fowl (cf. Pappenheimer [22]). In only one case (that of fowl 651) did I see slight leukostasis in the lumbar nerve. The ovary, testes and ganglions were found to be free from leukostasis.

Hemorrhages, particularly in the muscles, are frequently seen post mortem. Secondary parenchymatous degenerative changes, particularly marked in the kidney, are common, but have not as yet been studied closely.

Comment.—A progressive growth of lymphoid erythroblasts appears to be the essence of erythroleukosis. It would seem that the bone marrow is the first organ where the stasis caused by the multiplying lymphoid cells becomes manifest. This is then followed by a similar change in the spleen and in the liver and involvement of the other

22. Pappenheimer, A. M.; Dunn, L. C., and Cone, V.: J. Exper. Med. **49**:63 and 87, 1929.

organs. This sequence of events is probably connected with the rate and character of the flow of blood in various organs, leading to retention and multiplication of cells.

CASE HISTORIES

The histories of a few cases will be reported in illustration of erythroleukosis. The first two of these cases are typical as described and require no further comment.

Erythroleukosis of Erythroblastic Type.—Fowl 328 weighed 650 Gm. on July 20; it was inoculated intravenously with an emulsion of organ of fowl 268 M. On August 9, the white blood cells numbered 40,800 and the red blood cells 2,060,000; the differential count was normal (see table 2) on September 27, and 17. On October 3 the comb was slightly pale, but the blood smear appeared normal; on October 4 both red and white cells were decreased in number, and the blood smear showed pronounced erythroleukosis (see table 2). The bird died following the removal of 9 cc. of blood from a vein of the wing.

Postmortem examination showed a moderately emaciated fowl, with large amounts of clear, yellowish fluid in the peritoneal cavity. The thymus was not enlarged. Much clear, frothy liquid escaped from the lungs on slight pressure. The liver weighed 70 Gm.; it was brittle and reddish brown with a slight grayish hue. The spleen weighed 12 Gm. and was reddish brown. The kidneys were pale grayish brown. The mucosa of the duodenum was spotted with minute red areas. In the cecum numerous whip-worms were found. The bone marrow of the femur and tibia was grayish, and the consistency was increased. The humerus contained air.

Microscopic examination revealed that the capillaries of the liver were enormously distended and filled with lymphoid cells. In the portal tissue there was a mild infiltration with small lymphocytes. Myeloid metaplasia was absent. The hepatic cells were filled with small fat droplets; they appeared to be compressed and showed in some areas necrotic changes, caused apparently by leukostasis. The marrow was crowded with lymphoid cells located in apparently endothelial-lined spaces. Granulocytes and small lymphocytes were seen in very small foci only. The pulp of the spleen was likewise packed with lymphoid cells. Stasis was pronounced in the kidney and adrenal gland, but was remarkably absent from the ganglion attached to the adrenal gland.

Erythroleukosis of Lymphoid Cell Type.—On January 16 fowl 656 weighed 770 Gm.; it was inoculated intravenously with 2 cc. of plasma of fowl 365. On February 20, the number of white cells did not seem to be increased, but there were among them many large lymphoid cells (see table 2). Several erythroblasts, many of them of the early type, were seen on February 25; an enormous increase of large lymphoid cells (white blood count, 182,000), some with double nuclei, many with vacuolated cytoplasm, was observed, and occasional myelocytes. The bleeding time was greatly prolonged, the clotting of the blood being incomplete after one and one-quarter hours. (The blood of two control fowls clotted completely within three and one-half and four minutes.)

At autopsy the weight was 975 Gm. The breast muscle was strong, the comb and mucous membranes were very pale; there were numerous hemorrhagic areas in the muscles; a moderate amount of serous liquid exuded from the lung. The liver weighed 59 Gm., was grayish brown and moderately firm and showed numerous minute grayish streaks and spots. The spleen weighed 4 Gm., and was rela-

tively more enlarged than the liver; it was slightly firm, with a meaty consistency, pale and grayish; the follicles were just visible. The testes were small. The kidneys were pale and appeared slightly swollen. The bone marrow (of the femur and tibia) was pale, uniformly grayish brown, resembling the spleen in color. The consistency appeared to be slightly increased. Several minute hemorrhagic spots were found in the mucosa of the duodenum and to a less extent in the small intestine proper. There were several whip-worms in the cecum. The cecal follicles were much swollen, and their surface showed hemorrhagic spots.

Microscopically, the bone marrow was almost entirely composed of densely packed lymphoid cells. The capillaries of the liver were studded with similar cells, and in this organ there were a few small perivascular lymphocytic accumulations. In the pulp of the spleen, lymphoid cells were abundant. There were pronounced leukostasis in the kidney and marked stasis in certain areas of the thymus, duodenum and cecal follicle. In the latter two organs coccidia were numerous.

Erythroleukosis with Myeloid Leukosis.—Fowl 655 weighed 700 Gm. and was inoculated intravenously with 0.5 cc. of blood of fowl 365 M. on January 16. On February 28 a smear showed erythroleukosis (see table 2). On March 3 the breast muscle was strong, the bird active, the comb moderately pale with a slight yellowish hue; the white blood count was 33,000; the red blood count, 1,730,000; the hemoglobin, 25. The blood clotted completely in three minutes. In a smear were seen numerous erythroblasts, many of them primitive, several large lymphoid cells and occasional myelocytes. On February 6 there was no marked change. On March 17 a smear (see table 2) showed moderate anemia, the very immature forms wanting. The chicken died on March 24. It was emaciated, weighing 555 Gm. All the organs were pale; the thymus was very small. In the lung there were several areas containing mucopurulent material near the large bronchi, and also reddish-brown consolidated areas involving not over one tenth of the lung substance. The liver weighed 28 Gm.; it was reddish brown with a fine network of yellowish-gray areas. Its consistency appeared increased. The spleen weighed 1 Gm.; it was pale and grayish and the follicles were not visible. The bone marrow was uniformly pale grayish brown. The grayish color and increased consistency of this organ were the only appearances at gross examination suggesting leukemia.

On microscopic examination the marrow showed maximum hyperplasia formed by myelocytes, as well as by lymphoid cells. In the sinusoids there were also erythroblasts in all stages of maturation, with a dominance of the lymphoid forms. In the liver there were several small perivascular myelocytic foci, and there was slight stasis of lymphoid cells in the capillaries; some lymphoid cells formed also small extravascular foci. In the kidney and heart there was mild leukostasis. In the lung there were large areas of necrosis surrounded with inflammatory exudate.

Comment: This case is remarkable (1) for the disappearance of erythroblasts from the circulation about seven days before death, (2) for the inconspicuousness of the gross pathologic appearances in the liver and spleen, (3) for the intensity of the myeloid hyperplasia, which was combined with erythroleukosis, and (4) for the purulent infection of the lung, which might have been responsible for the atypical course.

Erythroleukosis with Slight Myeloid Involvement.—On January 16 fowl 681 weighed 790 Gm.; it was inoculated intravenously with 1 cc. of blood of fowl 465

after the blood had been treated with distilled water.[15] On February 28 a smear was suggestive of erythroleukosis (see table 2). On March 3 the comb was red with a faint yellowish hue, the breast muscle fairly strong, and the bird active. The white blood cells numbered 137,000 (see table 2); the red cells, 2,650,000. The hemoglobin was 42. In a smear erythroblasts were few; the picture was dominated by large lymphoid cells, several of which showed double nuclei and most of them vacuolization of the cytoplasm. On March 5 occasional mitotic figures and myelocytes were seen; the smear otherwise appeared as on March 3. On March 11 the white blood count was 315,000, the red blood count 1,040,000, the hemoglobin, 23; clotting of the blood was complete in ten minutes, and numerous large mononuclear cells were seen, similar to those observed in myeloid leukemia, some of them with large purple granules. Myelocytes and polymorphonuclear leukocytes were absent. Erythroblasts in all stages of development and primitive lymphoid cells were abundant. Mitotic figures were numerous. The fowl died on March 12.

At autopsy it was moderately emaciated, weighing 800 Gm. The comb was pale and slightly yellowish. The thymus was small and grayish. The lungs were pinkish and apparently normal. The heart was pale brown, with injected blood vessels. The esophagus and trachea were free. The liver weighed 71 Gm. and was distinctly grayish brown on the cut surface, with a fine grayish-white mottling. The spleen weighed 6.5 Gm.; it was slightly firm; the cut surface was grayish brown; the follicles were very minute. The bone marrow was uniformly grayish and resembled the spleen in color. The kidney was pale. The intestinal tract showed no marked abnormalities with the exception of the cecum, which showed a few minute hemorrhagic spots. The microscopic picture of the organs was that of pronounced erythroleukosis similar to that in case 328 described in foregoing paragraphs; myeloid involvement was slight and was localized in the liver and adrenal gland.

Erythroleukosis.—The development of leukemia in fowl 728 is well reproduced in table 2. The terminal blood picture suggested a myeloid complication, yet the microscopic picture of the organs was that of pure erythroleukosis.

Atypical (Aplastic?) Erythroleukosis.—Fowl 269 was first inoculated on Jan. 4, 1929, with material from fowl 177 E, with negative result. On Jan. 16, 1930, the bird (weight, 1,750 Gm.) was reinoculated with blood from fowl 365 M. The subsequent development of a blood picture suggesting erythroleukosis is given in table 2. The severe anemia, not observed to such an extent in typical cases of erythroleukosis, is noteworthy. Weakness of the leg was noted on April 3.

At autopsy, on April 8, the body weighed 1,450 Gm. and was moderately emaciated; the comb and organs had a pronounced icteric color. The liver was yellowish brown, and weighed 34 Gm.; the consistency appeared slightly increased. The spleen was small (0.75 Gm.). The bone marrow appeared to be partly yellowish gray and fatty, and partly grayish brown. The kidney was yellowish brown. The heart was somewhat dilated and flabby.

On microscopic examination the bone marrow showed advanced hyperplasia composed chiefly of densely packed young polymorphonuclear leukocytes and their precursors and to a less extent large lymphoid cells. In the capillaries of the liver, cells laden with yellowish-brown pigment were numerous. Leukostasis was indistinct and occurred to a very slight degree in a few capillaries only. In the periportal area there was a proliferation of large mononuclear cells with strongly

basophil cytoplasm and of myelocytes. In the spleen, which showed no specific involvement, pigment cells were abundant.

Comment: This case is noteworthy, for at gross examination there was no indication of the erythroleukosis suggested by the blood picture before death, and in the microscopic picture of the blood-forming organs the changes characteristic of erythroleukosis were indistinct. Since this and the similar case, in fowl 178, have not been transmitted it remains uncertain whether they were caused by the transmissible agent of the leukemia of fowls; their designation as erythroleukosis may be disputed.

Erythroleukosis of Erythroblastic Type Ending in Recovery.—The diagnosis in the case of fowl 715 was based on the examination of blood (see table 2).

Erythroleukosis with Sarcoma.—Fowl 675, on January 16, weighed 845 Gm.; it was inoculated intravenously with 0.5 cc. of blood of fowl 365 after the blood had been treated with distilled water. Blood smears taken repeatedly from February 20 until April 26 were negative. On April 29 a smear showed the picture of erythroleukosis. The bird died on May 6. On six successive days preceding death the fowl was fed with liver extract by Dr. Amano, who observed during this period a drop in the count of red blood cells to 1,000,000 and a simultaneous disappearance of the early erythroblasts. Hemorrhage from the liver was the immediate cause of death. The weight of the fowl post mortem was 1,070 Gm. The lung was pinkish, the heart pale and the kidney yellowish brown, but otherwise there were no marked changes. The liver was brittle and weighed 119 Gm.; it was motttled with numerous minute grayish-white nodules and contained several prominent whitish nodules varying in size from a few millimeters to about 3 cm., many of which were yellowish in the center. These nodes, unlike lymphoid tumors, did not emulsify easily and, when cut, formed small coherent particles. In the spleen, which was pale grayish and weighed 3.5 Gm., there was one tumor node of about 1 cm. across. A similar, but slightly firmer, tumor was found in the skin of the neck. The bones were somewhat soft, and in those containing marrow there was proliferation of fibrous bony tissue from the endosteum, in some places obliterating the cavity almost entirely. The marrow obtained from the femur was red; that from the tibia was greenish gray, the greenish color suggesting necrosis. The microscopic picture of the tumors of the spleen, liver and skin showed a great resemblance. They were formed by large, round, polymorphous, or less frequently somewhat elongated, cells and were diffusely infiltrated with leukocytes. In the tumor of the skin there was abundant reticulum. It contained numerous mitotic figures and infiltrated the muscle tissue diffusely. In the parts of the liver that were not grossly invaded by the tumor, lymphoid cells crowded the sinusoids and small foci of tumor cells and foci of myelocytes were seen. Leukostasis was not found in the kidney, adrenal gland and heart. and was indistinct in the spleen.

Comment: This and another similar spontaneous case (that of fowl 515), to be described in later paragraphs, give rise to the thought that erythroleukosis may be secondary to a tumor.

TABLE 3.—*Blood Counts in Spontaneous Erythroleukosis and Experimental Anemia*

Chicken	Date of Examination	White Blood Cell Count in 1000's	Red blood Cell Count in 1000's	Hemoglobin (Sahli)	Polychrome Erythrocytes	Polychrome Erythroblasts	Basophil Erythroblasts	Proerythroblasts	Primitive Lymphoid Cells	Same with Vacuoles	Mitotic Figures	Large Mononuclears	Promyelocytes	Myelocytes	Metamyelocytes	Polymorphonuclears	Mast Cells	Lymphocytes
						Spontaneous Erythroleukosis												
235	4/10	24.7	540	15	Abundant	Abundant	9	2	..	..	..	4	..	..	..	20	1	64
	5/10	Normal	.....	..	Abundant	Abundant	3	1	..	1	..	13	1	1	..	4	2	74
	6/25	Normal	.....	..	Abundant	Abundant	9	1	2	..	..	11	..	..	..	5	1	71
366	9/ 7	Normal	.....	..	.....	.....	..	..	..	..	..	13	..	..	1	54	..	32
	2/11	Normal	.....	..	Practically absent	Very few	11	2	8	10	1	17	..	..	..	4	1	46
	2/26	30	2,095	53	Many	Numerous	24	3	7	1	..	12	1	..	..	1	8	43
	5/ 2	Much increased	.....	..	Many	Abundant	53	9	12	1	5	3	..	2	3	10	..	2
441	5/21	930	500	13	None	None	..	..	95?	..	1	1	..	1	..	2	..	..
	5/26	975	465	..	None	None	..	..	96?	..	..	..	1	1	..	2	..	..
515	11/ 1	11	1,570	52.5	Abundant	Many	16	..	17	1	..	13	11?	..	..	29	5	8
						Experimental Anemia												
245	4/16	22.5	1,040	30	Abundant	Abundant	1	..	2?	..	..	10	..	..	..	33	4	50
292	12/12	40.5	740	Less than 10	Abundant	Few	..	..	..	..	..	23	..	..	..	68	4	5
	1/11	41	745	14	Abundant	Few	..	..	..	..	..	26	..	..	..	70	..	4

The relation of the erythroleukosis to the osteosclerosis in which the marrow is partly or wholly obliterated by bone needs further investigation.

SPONTANEOUS ERYTHROLEUKOSIS

There are no data available on the occurrence and incidence of spontaneous erythroleukosis among fowls. Those who have observed erythroleukosis did not distinguish it from myeloid leukosis or presumably considered it lymphoid leukemia.

Hare in cooperation with us found among 490 autopsies of fowls 22 spontaneous cases of leukosis, of which 3 resembled erythroleukosis. The data available on these cases are not sufficient for a full description. An "epidemic" of spontaneous erythroleukosis on a Pennsylvania farm is now being investigated by Biltz, Stubbs and myself.

Several cases of presumably spontaneous erythroleukosis have been observed in this laboratory among fowls not inoculated with leukemic materials. Their history will be fully described here.

Case of Spontaneous Erythroleukosis.—On Sept. 7, 1929, fowl 366 showed slight paleness, and on November 11, a relative lymphocytosis (for differential counts see table 3). On Feb. 11, 1930, a blood smear suggested incipient erythroleukosis. On February 26 the breast muscle was fairly well developed, the comb red, the weight 1,460 Gm.; the white blood cell count was 39,000, the red blood cell count 2,695,000, the hemoglobin 56; blood clotting was normal; the blood smear was as on February 11. On April 2, the blood smear was qualitatively erythroleukotic, but the number of immature red cells was scant. On May 2, a blood smear taken about one hour preceding death showed an abundance of early erythroblasts and lymphoid cells, some in mitotic division, and occasional myelocytes.

The weight of the fowl post mortem was 1,200 Gm. Its body was infested with a variety of ectoparasites. The skin and organs were pale; the breast muscle was fairly strong. The abdomen was much distended by clear, yellowish exudate. The liver was grayish brown and weighed 77 Gm.; it was studded with prominent yellowish-gray or yellowish-white nodules, varying in size from 1 to 3 mm. Only the upper third of the right side of the liver was free from such nodules. The spleen weighed from 3 to 5 Gm. and was uniformly pale grayish brown; its consistency was slightly increased. The bone marrow (femur) was pale and grayish brown; its consistency was likewise increased; it contained numerous minute nodules similar to those seen in the liver. The mucosa of the descending loop of the duodenum appeared to be swollen and hemorrhagic; similar areas were found also in the mucosa of the ascending part of the duodenum and in the small intestine.

On microscopic examination, pronounced stasis of lymphoid cells was observed in the liver. The nodules described were formed by myelocytes, many of them in the stage of mitotic division. In the upper part of the right lobe of the liver, which on gross examination appeared to be free from grayish nodules, the microscopic picture was that of erythroleukosis, with only one small myelocytic focus. The hyperplasia of the marrow was extreme, partly (about one-third) myelocytic and partly (about two-thirds) lymphoid. In the other organs, including the spleen, only stasis of lymphoid cells was noted, to a varying degree.

Comment: The qualitative blood picture revealed erythroleukosis at a time when the number of red cells was not appreciably diminished and when the amount of hemoglobin was sufficient. These observations indicate that spontaneous erythroleukosis, like transmitted erythroleukosis, does not develop from preexisting anemia. Temporary improvements in the qualitative blood picture have likewise been observed among the birds inoculated with leukemic material.

Spontaneous Erythroleukosis with Sarcoma.—Fowl 515, a barred Plymouth Rock capon, on November 1, appeared ill, showing weakness of the leg. The white blood cell count was 11,000 and the red blood cell count 1,570,000; the hemoglobin was 53. In a blood smear numerous polychrome red cells and polychrome erythroblasts, a few basophil erythroblasts and occasional primitive lymphoid cells were seen (table 3). Three days later the fowl was found dead.

Post mortem the weight was 2,600 Gm.; the breast was fairly well developed; there was much fat in the abdomen and in the subcutaneous tissue; the thymus was small. The heart was studded with grayish, moderately firm, prominent nodules, from 1 to 4 mm. in diameter. About two thirds of the lungs was consolidated, yellowish gray and adherent to the wall of the chest. The liver weighed 103 Gm. and was reddish brown with a slight grayish hue containing four large (from 1 to 2 cm.), and several smaller, grayish nodules. The spleen weighed 7.5 Gm., was soft, red and slightly grayish, and the follicles were prominent. The kidney was soft, brown and slightly grayish. In the left caudal lobe there was a tumor node, about 2.5 cm. in diameter, and a tumor about twice this size infiltrated the perineum. The marrow of the femur and tibia was reddish brown and distinctly grayish, and its consistency was increased.

On microscopic examination some capillaries of the marrow were filled chiefly with lymphoid cells, although not as densely packed as in most cases of erythroleukosis; others appeared to contain normal blood. Granulocytic tissue was scant. In the liver, as well as in the spleen, there was from mild to moderate stasis of lymphoid cells. The microscopic picture of the tumor itself was that of a sarcoma exhibiting great variability of the neoplastic cells from elongated or spindle-shaped forms to the large, round or polygonal type. There was mild, but distinct, stasis in the kidney and adrenal gland. In the latter organ there were also large foci formed by myelocytes and young polymorphonuclear leukocytes.

Comment: The observation of spontaneous erythroleukosis in association with tumor suggests the possibility that erythroleukosis might be secondary to tumor. The data available do not permit any definite conclusion with regard to this possibility. A similar case (that of fowl 675) has been described.

Atypical Leukemia Associated with Osteosclerosis.—On Sept. 21, 1929, fowl 441 weighed 1,150 Gm. On October 2, about 0.3 cc. of tar was injected into the marrow of the left femur; the marrow at the same time was partially destroyed. On November 22 the cavity of the right femur was cleaned with a solution of sodium hypochlorite and filled with gutta-percha rods. A blood smear of Feb. 25, 1930, appeared normal. On May 21 (see table 3) the circulating blood appeared to be flooded by white cells, exceeding the red cells in number (the white blood count was 930,000 and the red blood count 560,000). The neoplastic

cells resembled large lymphocytes. Mitotic figures were numerous. There were no transitional forms in any direction to establish the potentiality of these cells. On May 28, the bird was dying and was killed.

At autopsy, the chicken was moderately emaciated, weighing 1,650 Gm. The liver weighed 40.5 Gm. and was uniformly pale grayish brown. The spleen had a similar color and was much enlarged (8.5 Gm.); the follicles were just visible. The left femur was almost entirely ossified; the small amount of marrow that could be obtained was pale grayish. The right femur was likewise ossified, enclosing the inserted gutta-percha sticks. The process of ossification in the tibia was much advanced, but somewhat less complete than that of the femurs. The humerus contained air. The cavity of the ribs was likewise narrowed. The heart was pale grayish and dilated, and measured 3.5 by 5 by 1.7 cm. The parathyroid gland appeared normal. The intestinal tract seemed free from parasites with the exception of one ascaris in the distal part of the duodenum. The cecal follicles were much swollen and reddish on the surface. They measured 1.2 by 0.5 by 0.6 cm.

On microscopic examination, the liver showed pronounced stasis formed by cells closely resembling the lymphoid cells of erythroleukosis, from which they could not be differentiated on morphologic grounds. The character of these cells as seen in smears prepared from this organ was similar to that of the cells seen in erythroleukosis. In the bone marrow there was extreme hyperplasia, formed almost exclusively by similar cells; only a few foci of myelocytes were seen. The microscopic picture of the spleen likewise resembled that seen in severe erythroleukosis, and stasis was marked in the lung, heart muscle and kidney. Attempted transmission to twelve fowls has been unsuccessful.

Comment: The case described is unique. The pathologic picture was that of osteosclerosis with severe erythroleukosis, but the blood picture was dominated by medium and large cells resembling lymphocytes, exceeding the erythrocytes in number. Erythroblasts were absent. Most remarkable was the absence of pronounced basophilia and of nucleoli in most of the leukocytes of the blood. The lymphoid cells that formed the stasis in the liver had, on the other hand, the morphologic character of the early forms of lymphoid erythroblasts.

SPONTANEOUS GRAVE ANEMIA UNASSOCIATED WITH ERYTHROLEUKOSIS

A grave anemia of fowls was recently described in England by Bedson and Knight.[4] This anemia is associated with an enormous increase of lipochrome in the circulating blood, causing a yellowish discoloration of the skin and internal organs. The bone marrow shows erythroblastic hyperplasia, but in some instances it is replaced by bone. The mononuclear phagocytes of the spleen and liver are filled with blood pigment. The constant absence of stasis of lymphoid cells in the liver and other organs appears likewise to differentiate this condition from erythroleukosis.

This anemia appears to be chronic and more benign than erythroleukosis, and its causative agent produces only transient anemia when passed from sick to healthy fowls.

Subsequently Bayon [23] described thirty similar cases, which occurred only in hens of one breed (white Leghorn). All the affected hens were found to harbor a minute cestode, *Davaina proglottina,* in the duodenum. This parasite, Bayon assumed, creates a predisposition to an infection with a hypothetic endocellular microbe ("microplasm"). Osteosclerosis was likewise found by him in several instances. The formation of osteoid tissue was in his opinion a process secondary to the invasion of the marrow.

The contributions of Bedson and Knight and Bayon reached me when the material presented here was about to be reported. It would seem that none of the cases described here as erythroleukosis, except perhaps a few atypical cases mentioned, fall within their definition.

Case of Grave Anemia.—A white leghorn hen, no. 235, was received through the courtesy of Dr. Hare on April 10. The hen was well nourished and active, but very pale and yellowish, and its body was full of lice. The examinations of the blood (table 3) showed severe anemia. The condition of the bird seemed unchanged until June 26 when 12 cc. of blood was drawn from the neck for transfer. Death occurred three days later. The bird was very fatty, pale and yellowish. The liver was fragile, yellowish and mottled with small brownish areas. The spleen was brown and about twice the normal size. The marrow of the femur and tibia was pale grayish brown. On microscopic examination, only very slight stasis was found in the liver, which showed large areas of necrosis, diffuse degeneration and small foci of granulocytes (young polymorphonuclear leukocytes and myelocytes). The bone marrow was hyperplastic, but the hyperplasia was chiefly granulocytic and lymphocytic. Tranfers of an emulsion of organs to six fowls and of blood to four fowls had negative results.

Comment: Cases such as this have been described by Ellermann as "simple anemia," which he assumed was caused by the leukemic virus and represented an aplastic variety of erythroleukosis. This idea is not well supported. Erythroleukosis seems to be a well defined type of anemia from which cases such as that just described should be separated until their etiologic relationship is proved.

EXPERIMENTAL ANEMIAS IN THE FOWL

The numerous studies on the experimental production of anemia of the fowl will not be reviewed because in none of them is the development of erythroleukosis described. It seemed desirable to reinvestigate the possibility of producing erythroleukosis by prolonged bleeding and by the administration of blood poisons.

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23. Bayon, H. P.: Parasitology **21**:339, 1929.

Kasarinoff [24] did not support his assertion that a leukemic blood picture may be produced in the fowl by the administration of blood poisons by examination of the blood-forming organs, nor has it been confirmed by similar studies of other investigators.

Anemia Produced by Bleeding.—After heavy bleedings (from 20 to 50 cc.) there was a transient appearance of polychrome erythrocytes in the circulation. The capacity of the fowl to regenerate erythrocytes on repeated bleeding appears to be so powerful that the attempt to produce erythroleukosis by this means has been abandoned. The possibility that continuous loss of small amounts of blood over a longer period of time would result in erythroleukosis is conceivable, though not probable.

The history of one fowl is briefly given as an example of these investigations.

Fowl 315, on July 25, weighed 1,090 Gm. From November 14 until December 14 a total of 389 cc. of blood was removed in fourteen sessions. The fowl died three days after the last bleeding from an intercurrent purulent infection of the respiratory tract. Preceding the bleedings (July 25) the red blood count was 2,250,000, and three days after the last bleeding it was 2,390,000. The microscopic examination of the femoral marrow, liver and spleen did not show any indication of erythroleukosis.

Anemia Produced by Injection of Pyrodine.—Pyrodine (acetylphenylhydrazine) was found effective in producing erythroblastic hyperplasia of the marrow, as well as in promoting the appearance of erythroblasts in the peripheral circulation. Unlike the blood picture in erythroleukosis, that in pyrodine poisoning is dominated by polychrome red cells and maturing erythroblasts. The hyperplasia of the marrow is erythroblastic, with numerous transitional forms to erythrocytes, and not lymphoid as in erythroleukosis. In one case (that of fowl 245) the hyperplasia of the marrow had some resemblance to that in erythroleukosis, but in no instance was stasis of lymphoid cells in the liver or spleen observed. In the capillaries of the liver pigment-laden Kupffer cells are abundant, and similar cells are likewise numerous in the spleen. As an example of this series of experiments the histories of two fowls are given.

On June 22, 1929, fowl 292 weighed 837 Gm. The white blood cell count was 53,000; the red blood cell count, 2,890,000. On June 28 and July 25 the fowl was given ten injections of pyrodine (from 0.5 to 1 cc. of saturated watery solution) subcutaneously. In addition, from November 29 until Jan. 18, 1930, twenty-four intravenous injections of pyrodine were given. The first dose was 2 mg. and was increased subsequently to from 10 to 100 mg. The blood count twenty-four hours after the fourth injection (100 mg. on December 4) was: red blood cells, 835,000; white blood cells, 23,500. In a smear polychrome red cells and erythroblasts were

24. Kasarinoff: Folia haemat. **10**:391, 1910.

numerous, but early erythroblasts were scant. Two days later there was an improvement in the blood picture, and 100 mg. of pyrodine was injected, and doses of similar size were administered until death occurred. The extent of rapid regeneration is illustrated by the following figures: December 12, twenty-four hours after injection of 80 mg. of pyrodine, the red blood cell count was 740,000; two days later, it was 1,530,000, and four days later the blood smear appeared normal. On December 31 the red blood cell count was 440,000 and the hemoglobin was 10; on January 14, the red blood cell count was 745,000 and the hemoglobin 14. Death occurred twenty-four hours after the last injection. The fowl was pale and emaciated. The liver was chocolate-brown and very slightly enlarged; the color of the spleen was similar to that of the liver; it was not enlarged. The marrow was reddish brown, soft and juicy. Numerous ascarides were found in the duodenum. In the capillaries of the liver there were numerous brown, pigment-laden Kupffer cells. Similar cells were abundant in the pulp of the spleen. The bone marrow was very hyperplastic; the hyperplasia was mainly erythropoietic, with numerous transitional forms to erythrocytes and to a lesser extent granulocytic. The granulocytes were represented chiefly by young polymorphonuclear leukocytes. Fat was wanting. Leukostasis was not found in the blood-forming organs, the adrenal gland and the heart muscle.

Fowl 245 was killed two days after two intravenous injections of 20 and 40 mg. of pyrodine, given with an interval of twenty-four hours. The blood counts before death were: red blood cells, 1,040,000; white blood cells, 22,500; hemoglobin, 20. Polychrome erythrocytes and erythroblasts were in enormous numbers in the circulating blood, but few basophil erythroblasts were seen. The body was that of an apparently healthy, somewhat fatty fowl. The liver was yellowish with some chocolate-brown hue; it was of normal size. The spleen was purplish red, of about normal size; the follicles were prominent. The bone marrow of the femur was reddish brown, somewhat fatty and interwoven with numerous bony spicules. In the liver and spleen the appearances were somewhat similar to those in the same organs of fowl 292. The pulp of the spleen was studded with pigment-laden cells; the capillary mantle of Schweigger-Seidel, however, which has been maintained to be a part of the "reticulo-endothelial system," [25] was apparently free. About one half of the marrow was fat, but the remaining part was composed of lymphoid cells similar to those seen in erythroleukosis, with many of them in mitotic division.

The effect of ricin and of tolylenediamine was studied in a similar manner in three fowls each, and of saponin in four other fowls. Erythroleukosis did not result from these procedures.

Comment: It would seem that within a few days the fowl is capable of regenerating its entire volume of blood when it has been gradually destroyed by pyrodine and can repeat the process a number of times before this function is overcome.

The appearance of early erythroblasts in the circulating blood of anemic fowls is not surprising. The blood-forming organs of the lower vertebrates would seem less capable of retaining immature erythrocytes than is the marrow of man, for in the circulating blood of the lower

25. Staemmler, M.: Virchows Arch. f. path. Anat. **255**:585, 1925.

vertebrates multiplication and maturation of erythroblasts may even be physiologic (cf. Freidsohn [26] and Dawson [27]).

Characteristic of the anemia produced by pyrodine in contrast with erythroleukosis is the absence of leukostasis in the organs and the erythroblastic hyperplasia of the marrow with pronounced maturation. This is manifested by the character of the sinusoids, which contain mature erythrocytes in the central parts and erythroblasts near the periphery of the lumen.

COMMENT

Nature of Erythroleukosis.—The data presented on the transmissible leukosis of fowls are well explained by the assumption of a stem cell common to both erythroblasts and myeloblasts (lymphoid cell of the marrow, according to Pappenheim).[28] This cell in all probability resembles the large lymphocyte (cf. the case of fowl 441), but is not necessarily identical with it. It may mature in either of two directions, depending on the chemical or physicochemical factors created chiefly by their relation to blood channels (Hoogland [29]). Timofejewski and Benewolenskaja [30] incubated lymphoid cells from acute "myeloblastic" leukemia of man and observed their maturation into myelocytes on the one hand and into erythroblasts on the other hand.

The erythroleukosis and myeloid leukosis of fowls appear to consist of a tumor-like growth of immature blood cells deriving from this stem cell, the maturation of which is arrested at an early stage. The regeneration of the blood cells is unlike that seen under physiologic conditions (see Sabin [7]). There is no evidence of a proliferating endothelium, whereas mitosis among the primitive cells is abundant. The frequent observation that the larger blood vessels show no marked increase of lymphoid cells, but that the capillaries are studded with them, points to some factors directed toward a retention of immature cells in the capillary bed.

Ellermann rejected this conception as lacking a basis of observed fact. He assumed that erythroleukosis is similar to pernicious anemia in man, and that both a hypothetic toxin destroys the hemoglobin-containing cells, but spares the "erythrogonies." [31] The data presented

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26. Freidsohn, A.: Arch. f. mikr. Anat. **75**:435, 1910.

27. Dawson, A. B.: Anat. Rec. **45**:177, 1930.

28. The lymphoid cells of the marrow are assumed by Naegeli and by Hirschfeld to be myeloblasts and by Helly to be erythrogonies.

29. Hoogland, H. J. M., in von Heelsbergen, T.: Handbuch der Geflügelkrankheiten, Stuttgart, Ferdinand Enke, 1929, p. 474.

30. Timofejewsky, A. D., and Benewolenskaja, S. W.: Arch. f. exper. Zellforsch. **8**:1, 1929.

31. Helly's term for lymphoid cells with only erythroblastic potencies.

in this report, however, show that, unlike secondary anemia, erythroleukosis is marked by scant destruction of blood in early cases, and by the appearance of primitive cells in the circulation while there is yet an adequate supply of hemoglobin-containing erythrocytes.

Diagnosis of Erythroleukosis.—The diagnosis of erythroleukosis is based on (1) the appearance of early erythroblasts and lymphoid cells in the blood stream, (2) lymphoid cell hyperplasia of the marrow and (3) stasis of lymphoid cells in various organs, particularly in the liver and spleen. Cases of "simple anemia" with polychrome red cells and late erythroblasts in the circulation, but without abnormalities in the blood-forming organs, have not been proved to represent an "aplastic" variety of erythroleukosis, as assumed by Ellermann; their relation to the transmissible agent of the leukemia of fowls requires further investigation.

Relation of Erythroleukosis to the Anemias of Man.—Henschen [32] called attention to the similarity of erythroleukosis to the "anemia infantum pseudoleucemia" of von Jaksch. Common to both are the large number of erythroblasts, the enlarged spleen and the dark, but not invariably hopeless, prognosis. Other characteristics of the von Jaksch anemia, such as family occurrence, enormous increase of nucleated red cells after splenectomy, therapeutic failure of splenectomy and liver diet, efficiency of treatment with iron [33] and changes in the bones, have not thus far in the bird been systematically investigated. Extramedullary hematopoiesis seem to occur more readily in anemias of children than in those of adults, and occasionally it may assume a tumor-like growth (Brannan [34]).

Ellermann attempted to coordinate erythroleukosis with pernicious anemia in human beings (cf. also McGowan [3]). In both, maturation of erythrocytes is disturbed at an earlier age than in von Jaksch anemia (cf. Meyer and Heineke [35]). Phagocytosis of erythrocytes may occur in erythroleukosis, but is uncommon (Ellermann [1]). The similarity seems to be the closest to fetal erythroblastosis (von Gierke [36]) and to atypical forms of grave anemia, such as are described by Ferrata and de Negreiros-Rinaldi,[11] in which the mature forms of erythroblasts are absent from the circulation, whereas their basophil precursors appear in considerable amounts.

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32. Henschen, Folke: Arch. f. wissensch. u. prakt. Tierh. **43**:203, 1917.

33. Dr. Amano has failed to observe any change in the course of erythroleukosis after the administration of liver extract to six fowls.

34. Brannan, D.: Bull. Johns Hopkins Hosp. **41**:104, 1927.

35. Meyer, E., and Heineke, A.: Deutsches Arch. f. klin. Med. **88**:435, 1907.

36. von Gierke, E.: Virchows Arch. f. path. Anat. **275**:330, 1930.

SUMMARY

Erythroleukosis of the fowl is a disease characterized mainly by (1) severe anemia, with the appearance of lymphoid erythroblasts in the circulation, (2) splenomegaly and (3) the accumulation of cells resembling lymphocytes, presumably precursors of erythrocytes, in the capillaries of the organs, particularly of the bone marrow, liver and spleen.

At the beginning of erythroleukosis, erythroblasts usually appear in the peripheral circulation while there is still an adequate supply of erythrocytes and hemoglobin. In contrast with secondary anemias, the number of polychrome erythrocytes in the circulating blood in proportion to the number of early erythroblasts is not considerable.

Erythroleukosis has been produced by inoculation of blood, organ suspension or Berkefeld filtrate of fowls with myeloid leukosis or erythroleukosis. It occurs spontaneously, but its natural mode of transmission is unknown.

The anemia induced by repeated bleedings or by the administration of pyrodine differs from erythroleukosis mainly by the relatively small number of early erythroblasts in the circulation and by the absence of leukostasis in organs.

CARTILAGE IN A SALIVARY GLAND*

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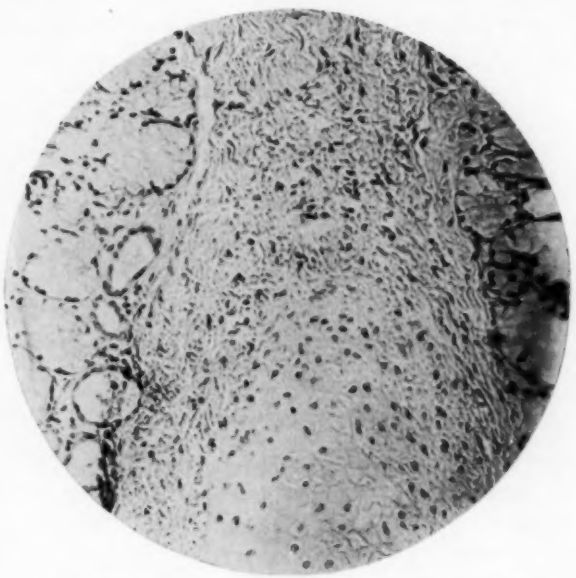
OMAHA

In an article on mixed tumors of the palate, the conclusion was reached that the theory of embryonic displacement appeared to be the most probable explanation of these tumors, particularly as compared with the hypothesis that the abnormal elements of connective tissue in



Cartilage in the framework of a salivary gland.

them are derived from glandular epithelium by metaplasia.[1] The specimen reported here, which was received some time after the publication of that article, is of interest in this connection. The material was removed from the mucosa of the pharynx because of local swelling following tonsillectomy in the belief that the enlargement was the result of hypertrophy of the lymphadenoid tissue, a belief which was substantiated by histologic examination. However, in a small portion of underlying salivary gland that formed part of the material there was observed the feature shown in the photomicrograph—a nidus of cartilage located

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* From the University of Nebraska College of Medicine.

1. Eggers, H. E.: Mixed Tumors of the Palate, Arch. Path. **6**:378, 1928.

in the interlobular connective tissue, entirely unassociated with epithelial elements, even in sections cut at varying depths, and too small even to produce deformity of the connective tissue septum.

It would, of course, be possible to explain the occurrence of this cartilage by the theory of metaplasia, but this would involve the complete disappearance of the original epithelium and the development of the process without the formation of a tumor. It would seem much more probable that the cartilage was the result of inclusion in the connective tissue framework of the gland of a cartilage rest, in this case without the other elements that contribute to the structure of the mixed tumors, and, until the time of its accidental removal, with an absence of the unknown factors that lead to the development of a tumor.

THE OCCURRENCE OF UNIDENTIFIED SPORANGIA IN THE TONSIL CRYPTS*

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In about 50,000 tonsils that I have examined during the past thirty-five years, two cases stand out as unique in that the tonsillar crypts contained spherical bodies having hyaline walls and packed full of spores, mostly in an unripe stage. The bodies varied from 30 to 300 microns in diameter. Larger, irregular, partly collapsed or shrunken sporangia containing ripe spores were also present. The histories of the patients, with the diagnoses given at the time, are as follows:

E. B., a woman, aged 21, had frequent sore throat. A tonsillectomy was performed. The pathologic diagnosis was: chronic hyperplastic tonsillitis; increase of stroma; hyperkeratosis; colonies of mouth organisms in the crypts; mold in one crypt, probably *Aspergillus*, tonsillar mycosis.

M. S., a girl, aged 11, had frequent sore throat. A tonsillectomy was performed. The pathologic diagnosis was: chronic hyperplastic tonsillitis; increase of stroma; hyperkeratosis; colonies of mouth organisms in the crypts; abscess of a crypt; numerous colonies of a moldlike organism in the keratohyalin of one crypt, without radiation, but surrounded by a hyaline zone; tonsillar mycosis.

The extreme rarity of this observation has always been a matter of interest to me, and the identification of the organisms in the two cases, apparently identical, an affair of some concern. That the structures were sporangia seemed evident, and this view has been concurred in by all who have seen the preparations. No hyphae were apparent in any of the serial sections made of these tonsils. *Rhinosporidium seeberi* has been the organism lately most frequently considered; and at one time a diagnosis of infection with this organism was tentatively made. Recently, however, a case of infection with *Rhinosporidium seeberi* came into the laboratory, and the study of this case makes it evident that the sporangia in the two cases of tonsillar infection in question are different from those of *Rhinosporidium*. Photographs of slides from the tonsil were submitted for an opinion to Prof. W. H. Taliaferro of the University of Chicago, whose first impression was that they were *Rhinosporidium*, but on my sending a slide to him, he reversed his opinion on the ground that the cytoplasm did not look like that of *Rhinosporidium*, and that the wall around the body did not appear to be the chitinoid covering seen in that parasite. Moreover, he thought

* Submitted for publication, Oct. 22, 1930.
† Dr. Warthin died on May 23, 1931.

that the hyaline wall around the body showed a certain number of nuclei, suggesting that the wall was laid down by the host and was not part of a parasitic cyst. The absence of nuclei within the parasite he also regarded as evidence that it was not *Rhinosporidium*. Professor Taliaferro showed the slide to Professor Ashworth, during a visit of the latter to Chicago, who agreed that the structures were not *Rhinosporidium*. Dr. C. V. Weller, whose careful and thorough study of a case of infection with *Rhinosporidium* has appeared recently,[1] likewise did not think that these tonsillar parasites were *Rhinosporidium seeberi*, but that they were the

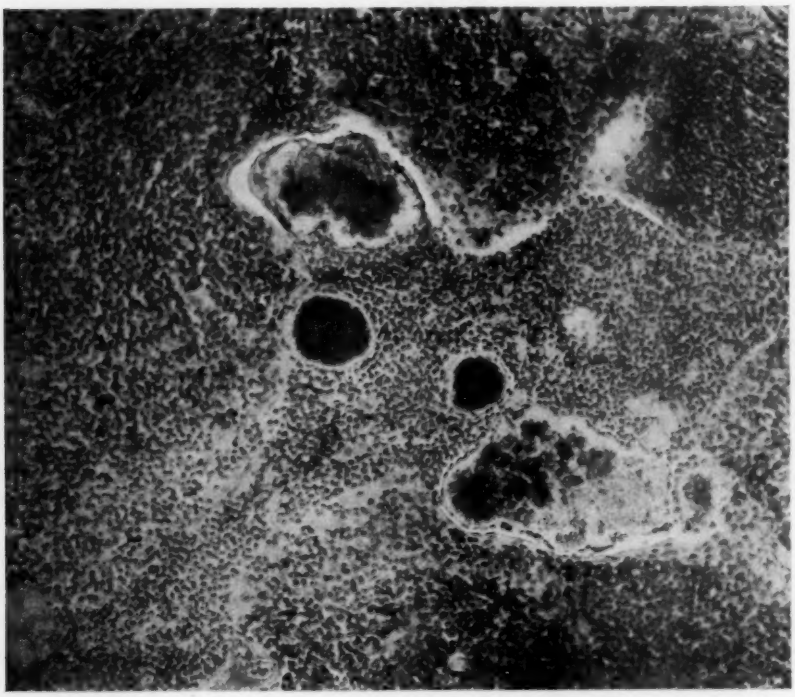

Fig. 1.—Low power view of an abscess of a tonsillar crypt, containing two sporangia with unripe spores and two partly collapsed and emptied ripe sporangia.

sporangia of some closely related organism. I have found nothing in the literature on the tonsil to throw any light on the identity of the organism in question.

As shown in figure 1, the organism consisted of a spherical body with a definite, but narrow, hyaline, nonnucleated capsule, around which was a layer of thin, flattened, squamous epithelium derived from the epithe-

1. Weller, C. V.: Rhinosporidium Seeberi: Pathological Histology and Report of the Third Case from the United States, Am. J. Path. **6**:721, 1930.

lium of the crypt. This layer contained the nuclei mentioned by Taliaferro, and was derived from the host and was not part of the parasite. It was evident that the structure had developed within the mucosal epithelium. The hyaline capsule was thinner, less hyaline and chitinoid than that of *Rhinosporidium seeberi,* but was well defined. It stained pinkish with eosin. Within the capsule was a thickly crowded mass of unripe granular spores, staining bluish with hematoxylin. Only an occasional nucleus could be made out. Two of this unripe form are shown in the figure. In addition there are shown two larger, irregular, partly

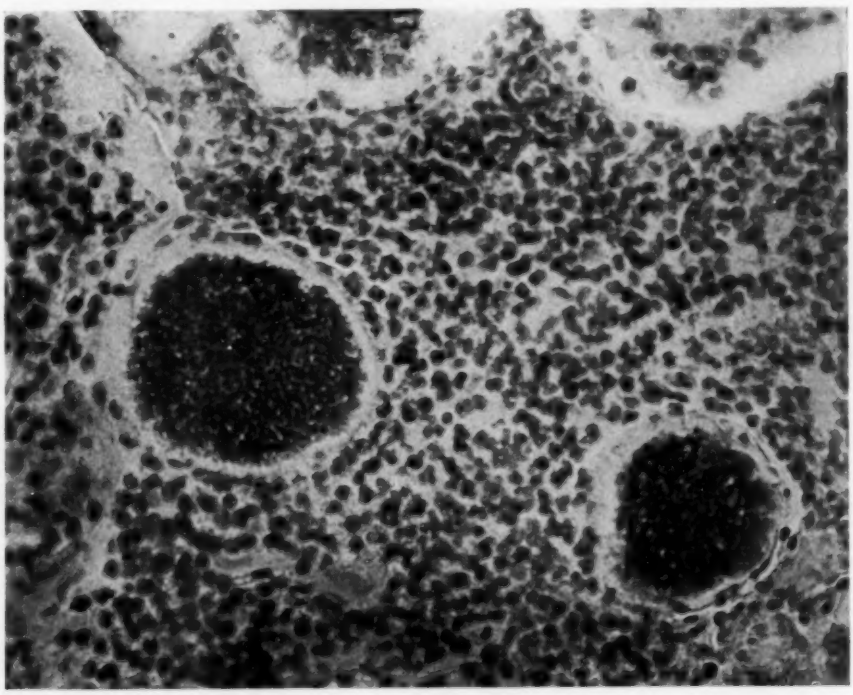

Fig. 2.—Higher power view of the unripe sporangia seen in figure 1.

collapsed sporangia, the spores of which had been in part discharged. Ripe spores and a finely granular material were contained in these collapsed sporangia. The structures were all embedded in the desquamated epithelium, which was heavily infiltrated with polymorphonuclear leukocytes and might have been termed a cryptic abscess. Beyond this there was no evident reaction of the tonsillar tissues to the presence of the parasite. None was found in the lymphoid tissue or stroma. The process was purely a crypt infection.

In figure 2 a view of the two spore-filled sporangia under higher power than in figure 1 is given. The hyaline capsule of the sporangium

with its applied layer of flattened epithelium is well shown. Comparing these sporangia with those of *Rhinosporidium,* the chief difference is in the thinner, less chitinoid capsule of the organism found in my cases. Whether this might be due to the difference in environment between an organism growing within the tissues of a nasal polyp and one growing in the epithelium of the tonsillar crypts cannot be answered. It is also of interest that both my cases occurred in women, whereas *Rhinosporidium seeberi* has been reported as occurring only in male patients.

In conclusion, it is considered important to record the unique observation in the tonsillar crypts of 2 of 50,000 tonsils examined in this laboratory, of sporangia of an unknown parasite, suggesting a close relationship with *Rhinosporidium seeberi.*

THE RÔLE OF THE ANTERIOR LOBE OF THE PITUITARY GLAND IN GROWTH

WITH SPECIAL REFERENCE TO THE TEETH AND MAXILLAE *

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It is apparent from a survey of literature on the endocrine glands that endocrinologists in general are of the opinion that glands of internal secretion markedly affect the growth, shape, size and placement of the dentition. With the exception of the work of such men as Erdheim,[1] Toyofuku [2] and Gies [3] as regards the effect of the parathyroid gland on the teeth, these opinions are in large part based on empiric and clinical reasoning only.

My own earlier studies [4] of a clinical nature in this connection reveal the fact that variations in form, placement, etc., are largely hereditary. Variations in density and spacing, as well as in the surrounding structure, are very much higher in all groups showing endocrine dyscrasias, but they fail to disclose any regularity in this direction, which would make such dental variations symptomatic of endocrine dyscrasias. The high percentage of variations in these groups, however, made it seem that there was some definite connection between the glands of internal secretion and the growth and development of the teeth. It is felt that perhaps in approaching this from the clinical point of view, one reason for the difficulty in obtaining such regularity was the inadequate symptomatology of endocrine dyscrasias; that is, it is one thing to diagnose a case clinically as of a certain endocrine type but an entirely different thing to know beyond a doubt that a dyscrasia of a particular gland is actually the cause of the symptoms presented.

For this reason, it has been deemed advisable to undertake from the experimental angle a study of this relationship. Owing to its

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* Submitted for publication, Oct. 3, 1930.

* From the Yale University School of Medicine.

* Dr. E. M. K. Geiling supplied me with the earlier extracts used and Dr. E. A. Sharp, of Parke, Davis & Company, those used in the later studies.

1. Erdheim, J.: Zur Kenntnis der parathyreopriven Dentin-Veränderung, Frankfurt. Ztschr. f. Path. **7**:238 and 295, 1911.

2. Toyofuku, T.: Ueber die parathyreoprive Veränderung des Rattenzahnes, Frankfurt. Ztschr. f. Path. **7**:249, 1911.

3. Gies, W. J.: Studies of Internal Secretions in Their Relation to the Development and Condition of the Teeth, J. Am. Dent. A. **5**:527, 1918.

4. Downs, W. G., Jr.: Studies in the Causes of Dental Anomalies, J. Dent. Research **8**:3, 1928.

generally accepted effect on growth, which has been adequately demonstrated clinically and experimentally,[5] the pituitary gland appeared to be the member of the endocrine series most likely to yield definite results in this direction. With that in mind, the following study was undertaken. As such an investigation covered, to a very great extent, uncharted ground, it was necessary to determine first, experimentally, what was the most likely means of influencing such growth, and second, what were the most clearcut methods for determining the rate and the direction of the effect, when obtained. In short, it was necessary to become thoroughly familiar with the field concerned.

Both of these preliminary studies have been done in the present instance in the belief that growth, as applied to any individual organ or member, is only a part of the general process of growth of the organism. The initial stages of the study fell then, naturally, into two divisions: investigations as to the general effects of the hypophysis on growth, and those concerned with measurements of the effects on the teeth and on other organs and tissues.

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5. Cushing, Harvey: The Pituitary Body and Its Disorders, Philadelphia, J. B. Lippincott Company, 1912. Dott, N. M.: The Syndromes of Experimental Pituitary Derangements, Abst., Eleventh Internat. Cong. Physiol., Edinburgh, 1923. Evans, H. M.: The Function of the Anterior Hypophysis, Harvey Lectures, Philadelphia, J. B. Lippincott Company, 1924, p. 212. Evans, H. M., and Simpson, M. E.: Antagonism of Growth and Sex Hormones of the Anterior Hypophysis, J. A. M. A. **91**:1338, 1928. Evans, H. M.; Cornish, R. E., and Simpson, M. E.: Potent, Sterile, and Low-Protein Extracts of the Growth Hormones from the Anterior Hypophysis, Proc. Soc. Exper. Biol. & Med. **27**:101, 1929. Flower, C. F.; Forkner, C. E.; Kellum, W. E.; Walker, A. T.; Smith, P. E., and Evans, H. M.: Separation of the Principle in the Anterior Hypophysis Affecting Ovulation, from that Controlling Body Growth, Anat. Rec. **25**:107, 1923. Korenchevsky, Vladimir, and Dennison, Marjorie, H.: The Influence of the Hypophysis on Metabolism, Growth and Sexual Organs of Male Rats and Rabbits: I. Influence of Extract of Hypophysis on Nitrogen Metabolism, Biochem. J. **23**:868, 1929. McLean, A. J.: Transbuccal Approach to the Encephalon, Ann. Surg. **88**:985, 1928. Putnam, T. J.: Separation of Growth-Promoting Hormone from That Inducing Premature Oestrus in the Anterior Pituitary Gland, Arch. Surg. **18**:1699, 1929. Putnam, T. J.; Teel, H. M., and Benedict, E. B.: The preparation of a Sterile Active Extract from the Anterior Lobe of the Hypophysis, Am. J. Physiol. **84**:157, 1928. Putnam, T. J.; Benedict, E. B., and Teel, H. M.: Studies in Acromegaly: VIII. Experimental Canine Acromegaly Produced by Injection of Anterior Lobe Pituitary Extract, Arch. Surg. **18**:1708, 1929. Reichert, F. L.: Effects of Anterior Pituitary Extract upon an Hypophysectamized Puppy, Proc. Soc. Exper. Biol. & Med. **27**:304, 1929. Smith, P. E.: The Disabilities Caused by Hypophysectomy and Their Repair, J. A. M. A. **88**:158, 1927. Teel, H. M.: Diuresis in Dogs from Neutralized Alkaline Extract of the Anterior Hypophysis, J. A. M. A. **93**:760, 1929. Teel, H. M., and Watkins, O.: The Effect of Extract Containing the Growth Principle of the Anterior Hypophysis upon the Blood Chemistry of Dogs, Am. J. Physiol. **89**:662, 1929. Zondek, B., and Ascheim, S.: Das Hormon des Hypophysenvorder lappens, Klin. Wchnschr. **7**:831, 1928.

EFFECTS ON GENERAL GROWTH

For the investigation of the effects on the general growth, it was felt that the small rodents, particularly mice, lent themselves most readily to the type of study desired, as they were easy to handle in large numbers and their entire life span was sufficiently short to be observable in the laboratory. For this reason, the initial stages of the studies were applied to these animals. Those employed were originally obtained from the excellent colony of Dr. C. C. Little at Ann Arbor, and all of those utilized were the descendants of this original group. In order to eliminate the factor of individual variation as far as possible, the animals were closely inbred throughout the existence of the colony, and as far as was practicable, litter mates were used in the various divisions of the studies involved.

One series of seventy-five mice was divided as follows: (1) a group (twenty-five animals) which was fed the anterior lobe of the beef hypophysis daily; (2) a group (twenty-five animals) which was fed liver in similar quantities daily, and (3) a group receiving the usual diet for these animals. As was the case for the previous studies,[4] in which dogs were used, although this group of investigations was continued for a period of almost six months, the results of this type of treatment were negative. The animals fed liver and those fed hypophysis gained at a slightly higher rate than did the control group. However, as between these two groups, there was practically no variation in increase in size, and it was felt that the only results observed were due to the somewhat higher intake of protein in the experimentally fed groups.

Attention was therefore turned to injection of the extract in the attempt to influence the growth of the animals. My earlier studies in which commercial extracts were used, while productive of some results, had not been sufficiently definite and clearcut to make it seem probable that the best results were to be obtained from this type of experimentation. This was likewise in line with the results of various other experimenters. For this reason it was determined to try a type of extract which could be followed in the laboratory. Evans [6] had very carefully detailed the preparation of an extract which in his hands gave excellent results in influencing the growth of mammalian forms. It was therefore determined to give this particular extract a careful trial. Beginning in October, 1928, I tried this type of extract on several series of animals (mice). The dosage, frequency of medication and other variables were experimented with from every angle. These animals were checked against untreated animals in some cases; in other

6. Evans (footnote 5, third reference).

cases, the control group received an extract of liver prepared in the same manner as the pituitary extract; and in still other series, the controls received physiologic solution of sodium chloride in similar quantities. In every series of experiments, it was found that the animals receiving the Evans alkaline extract, especially if its reaction was kept well on the alkaline side (p_H 7.6 to p_H 7.8), gained weight at a considerably more rapid rate than did the control littermates.[7] In many instances, this gain in weight was so marked as to appear almost phenomenal.

Quite by accident, as the result of the shipping of some animals that were being experimented on, it was found that if these animals

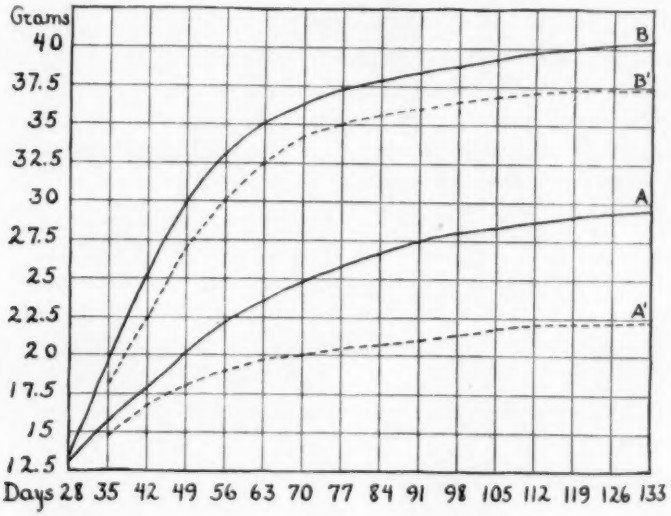

Fig. 1.—Changes in weight: *A*, of untreated controls; *B*, of animals treated by injection of alkaline extract of the anterior lobe of the pituitary gland; A^1 and B^1, of the same groups, computed on a fat-free basis.

had their food, or particularly their fluid, intake, sharply curtailed, the massive animals that received the extract of the anterior lobe lost weight at a tremendous rate and considerably more rapidly than did the control animals. This was carefully checked, and it was found that a considerable portion of the increase in weight in these animals was due to an increase of water in the tissues, and that the decrease was due to a rapid loss of this water. In order to analyze this, a new group

7. In the later studies, Evans' and Simpson's modifications of the original extract (footnote 5, fourth reference) were in part substituted, and in the later experiments on dogs, in a part of the experiments, an extract made according to Evans' last modification (footnote 5, fifth reference), made by Parke-Davis & Company, was used.

of animals was given similar treatment. Of these, four experimental and four control animals, two of each sex in each instance, were killed, first, at weekly intervals, and later every two weeks, and their bodies thoroughly ground and mixed and chemically analyzed for the presence of water, total nitrogen, total ash and fat, and curves plotted to represent the changes in these contents (figs. 1, 2 and 3).

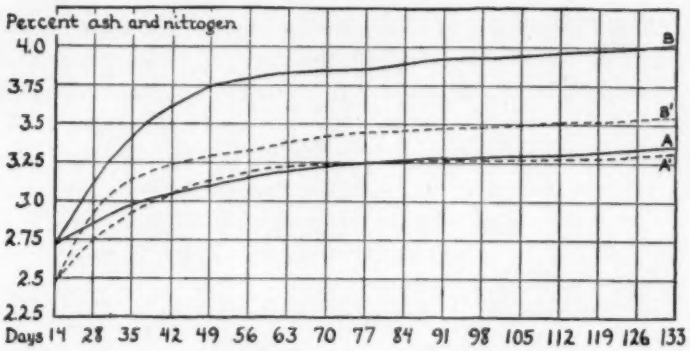

Fig. 2.—Changes in total ash and nitrogen contents ascertained at the periods indicated: *A*, ash content of control group; *B*, of the experimental group; A^1, nitrogen content of control group; B^1, of experimental group.

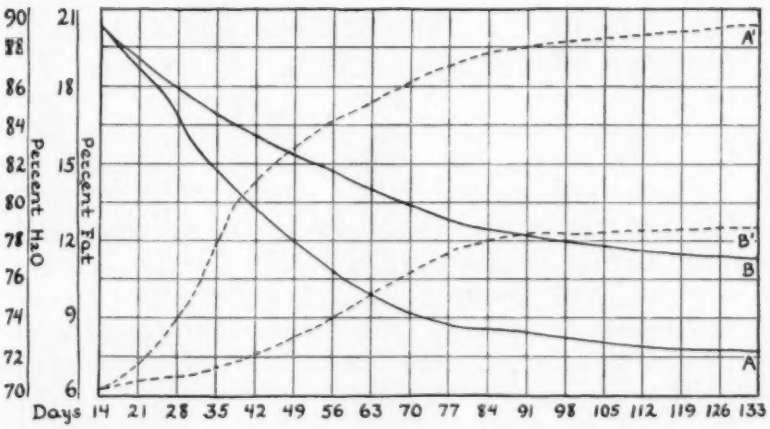

Fig. 3.—Changes in water and fat contents at the ages indicated: *A*, water content of controls; *B*, of animals treated by injections of alkaline extract of the anterior lobe of the pituitary gland; *A*, fat content of controls; *B*, of treated group.

Careful analysis of these results reveals that the animals receiving the alkaline extract of the anterior lobe of the pituitary gland gained in weight at a considerably more rapid rate than did their littermates; that the more rapidly growing animals given injections contained a

considerably higher water content at all levels and a somewhat complementary decrease in fat content; that the more rapidly growing animals given injections had a considerably higher ash content than did the control animals, and that the nitrogen contents of the two groups showed such a slight difference as to be considered negligible.

EFFECTS ON TEETH, LIVER AND OTHER ORGANS

An attempt was made to follow the method of Marshall [7a] in injecting trypan blue or naphthalene brilliant blue or one of the alizarin dyes to obtain definite lines of deposition of calcium in the teeth and to show

TABLE 1.—*Measurements of Teeth in Animals Receiving Injections of Extract of Anterior Lobe of Pituitary Gland, Compared with Measurements of Teeth of Untreated Controls*

Experimental Animal	Growth, Mm.				Averages
	1st Week	2d Week	3d Week	4th Week	
47	1.2	1.2	1.4	1.3	1.275
51	1.3	1.4	1.4	1.4	1.375
52	1.3	1.3	...	1.3	1.3
54	1.3	1.2	1.3	1.3	1.275
61	1.4	1.4	1.4	1.4	1.4
63	1.3	1.3	1.4	1.3	1.375
92	1.3	1.2	1.3	1.2	1.25
93	1.4	1.3	1.4	1.4	1.375
General average					1.328
Control Animal					
101	1.1	1.1	1.0	1.1	1.075
103	1.0	1.0	1.0	0.9	0.975
107	1.2	1.1	1.1	1.1	1.125
116	1.1	1.0	0.9	1.0	1.0
122	1.0	1.0	1.0	1.0	1.0
123	0.9	0.9	0.9	1.0	0.975
133	0.9	1.0	0.8	0.9	0.9
134	1.0	0.9	1.0	0.9	0.95
General average					1.000

the rate of growth in this manner. However, the results obtained were not at all satisfactory, and it is felt that no conclusions may be drawn from this portion of the study. On the other hand, by marking the constantly growing incisors with a fine thin disk on a dental mount and marking again at regular intervals, evidences as to differences in rate of growth of these teeth were obtained that were fairly satisfactory. By this method it was determined that the animals treated by injection showed a more rapid growth in the continuously growing incisor teeth than the control animals. In this series it was determined that the average rate of growth of the incisors was about 1 mm. per week in a normal untreated animal. In thirty-eight animals treated by injec-

7a. Marshal, J. S.: An Experimental Study, with Certain Vital Dyes, of the Persistent Teeth of the Albino Rat, J. Dent. Research **2**:1, 1920.

tion and studied in this manner not one showed this low rate of growth; the average rate of growth in these animals was 1.3 mm. per week.

The data in the table, giving the actual measurements of one group treated by injection and one group of controls, studied in this way for a period of four weeks, are typical of the rate of growth throughout.

Further than this, roentgenograms of both types of animals demonstrated that the mandibulae and teeth of the animals given injections were larger and of greater density than those of the littermate controls.

It was noted that in these animals there was a marked splanchnomegaly, in which the liver, in particular, was greatly enlarged, even out of proportion to the rate of increased growth. It was likewise apparent that the size of the internal female genitalia was markedly increased in these animals.

In August of 1929, similar studies were begun on dogs, and it was decided to study in addition to the effects of injection of the hypophyseal extract, those of hypophysectomy, on the growth of these animals. In earlier studies the temporal approach to the operation had been attempted with unsatisfactory results, and it was felt that in utilizing very young animals the results to be obtained would be even less desirable. For this reason, the transbuccal approach of McLean,[8] which offered wide access to the region, was decided on, and a number of dogs were the subject of these operations. In all, four successful operations were done, in two animals of each sex, each being about 10 weeks of age. Each of these animals was then grouped with two littermates of the same sex, one of which was handled as an untreated control and one of which received daily injections of the hypophyseal fraction affecting growth. The dosage in these animals was varied, being increased as the animals increased in age and size, until a maximum of 20 cc. daily was attained. Each of the twelve animals in this experiment was studied carefully at the outset, and its physical characteristics and the state of its dentition was noted at that time. The results obtained in each of the groups of three animals were consistent throughout, although varying slightly in individual cases. The dogs receiving the fraction promoting growth grew at a much more rapid rate than did the controls and were, in every sense, larger and heavier. Those that had been hypophysectomized increased in weight and size at a much slower rate than the controls and showed a considerable number of typical stigmas, in particular a myxedematous appearance.

In each case, also, the animals receiving the injections of the extract affecting growth shed their deciduous teeth and erupted the permanent ones at an earlier period than either of the other two types of animals,

8. McLean (footnote 5, reference 8).

while the hypophysectomized animals were far behind the controls in this respect. Further, the animals given injections had larger maxillae, the hypophysectomized smaller, which gave origin to differences in the position of the teeth (figs. 4 and 5).

One group of males was killed after six weeks of the experimentation, but the observations at autopsy were so slight as to be scarcely more than an indication. After fourteen weeks of the experimentation, one group of females was killed and carefully examined. Unfortunately, on the second day following, owing to a pulmonary infection, it was necessary to kill the second group of females. The results from these autopsies were sufficiently suggestive to indicate the direction of the results. After twenty-two weeks of experimentation,

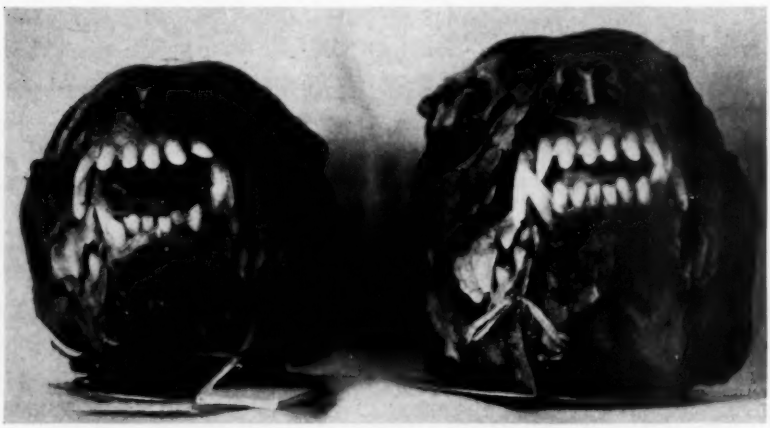

Fig. 4.—Front view (left) of the anterior teeth of a hypophysectomized male dog (no. 10), showing tumbling and "flaring" of the incisors and cuspids, and (right) of a male littermate (no. 11) treated by injection of alkaline extract of the anterior lobe of the pituitary gland, showing the larger maxillae, the spacing and the vertical position of the incisors and cuspids.

the death of one of the animals from advanced toxicosis made it necessary to kill and examine the remaining group. Reference to the accompanying charts will show the differences in rate of growth of the animals involved (fig. 6).

It is noteworthy that the gross changes in these animals were practically identical with those in the earlier series of mice. In each case the injections caused greatly accelerated growth, splanchnomegaly and acceleration in the dentition. In the hypophysectomized dogs, the changes were the opposite of these. Chemical studies on the dogs' tissues were not practicable, but in the case of both types of animals

the animals treated by injection contained, grossly, less fat than did the controls.

Microscopically, the livers of the injected animals showed larger cells, but not sufficiently so to account for the great increase in the gross size of these organs. This was likewise true of the uterus and tubes of the animals. In the case of both organs the reverse was true of the hypophysectomized animals. The uteri of the hypophysectomized

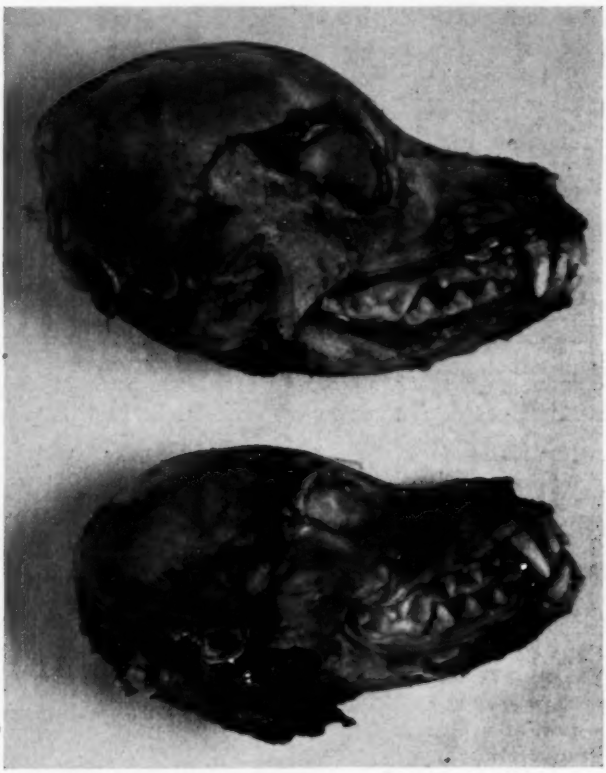

Fig. 5.—Lateral view of the heads of the animals shown in figure 4: above, an animal that had received injections of an alkaline extract of the anterior lobe of the pituitary gland; it shows frontal bulging, a larger head and jaws and a vertical position of the anterior teeth; below, a hypophysectomized animal, showing frontal flattening, smaller head and jaws and flaring of the anterior teeth due to a diminished arch.

animals were very infantile microscopically, as well as grossly, while those of the controls and of the animals treated by injection showed a marked increase in the endometrial glands and epithelium. Likewise, the ovaries of the hypophysectomized animals showed no evidence of advanced follicles, while those of the controls and of the animals treated

with the extract promoting growth showed many far advanced, but not ripe, follicles. The animals given injections showed a marked increase in stroma, over the controls, in each of these organs. Microscopic study of the teeth and investing tissues yielded negative results in each instance. No changes were to be observed in pulpal cells, ameloblasts, dentin, enamel or surrounding tissues. Neither were there differences in size between corresponding teeth in the different groups of these animals.

Throughout the lymphogenous organs, there was a noticeable increase in the size of the organs in the animals given injections, and microscopically this was found not to be due to an increase in the lymphocytes,

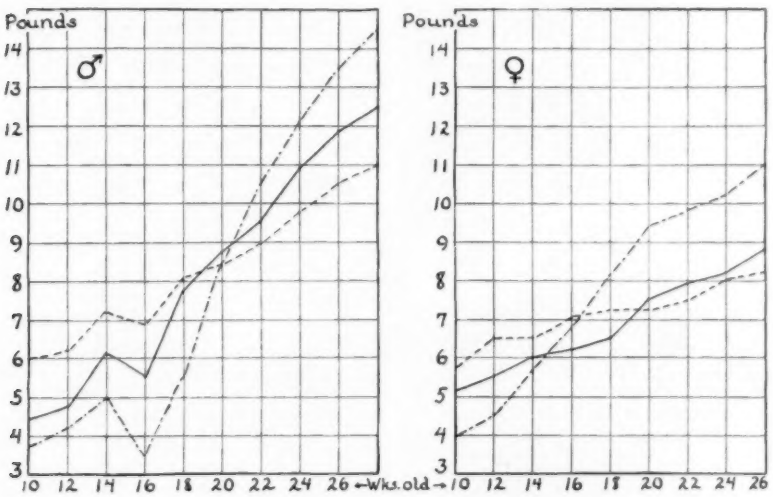

Fig. 6.—The chart at the left shows the growth curves of a litter of male dogs, carried under experimental conditions for twenty-four weeks; that at the right, the growth curves of a litter of female dogs, carried under experimental conditions for sixteen weeks.

but rather to a marked increase in the sustentacular tissue and to an edematous change in the organs. The liver cells showed necrosis, especially in the region of the central veins. This, with the microscopic changes in the kidneys and those in the lymphogenous organs, suggested the presence of some toxic change. What the cause of this change was, it would be difficult to determine. The method of preparation of the extract is one possibility. More likely, however, is the possibility that such greatly accelerated metabolism was itself productive of a toxicosis. Throughout all of the organs examined there was a varying apparent increase in the connective tissue elements in the animals

treated by injection, most marked in the lymphogenous organs, that is, the lymph glands, thymus and spleen. No change of this sort was noted in the salivary glands, pancreas or lungs, but a less marked one was present in the liver and kidneys.

Roentgenograms of the mandibulae of the mice showed an increase in size and density of these and of the continuously growing incisors in the animals given injections. There was likewise an apparent change in the dentition and the mandibulae of the dogs. In addition to this, in the dogs there was a marked difference in the size and shape of the head, especially noticeable in the frontal region (figs. 4 and 5).

CONCLUSIONS

There is in the anterior lobe of the hypophysis a substance that markedly affects the general growth of the organism. This effect is exercised in the direction of maintaining the water content of the tissues of the animal at a high level and in reducing the animal's tendency to put on fat. Its tendency is to increase the total mineral content of the tissues over a normal level for a given period of age. Its effect on the dentition of the animal is to markedly accelerate this process and to change the position of the teeth in the jaws, owing to a difference in the size of the jaws. Further, there is a probable toxic effect, evidenced microscopically in the liver, kidney and lymphogenic organs and due probably to the toxic products of too rapid stimulation of metabolic processes. There is likewise an apparent tendency for the growth-promoting hormone to stimulate especially the growth of the supporting tissue elements.

If these results are compared with those of Donaldson [9] and others, on the normal growth of rodents, the conclusion seems warranted that the effect of the extract of the anterior lobe of the hypophysis is to stimulate these animals in the early period of rapid growth (the third phase of Donaldson) to an even greater rate of growth and to somewhat prolong this period into the later, or fourth, phase. It also seems apparent that these effects are in no sense specific for the dental mechanism or inclined to be productive of specific anomalies of the teeth, but are exercised simply in the direction of rate and time of development, and that if there is a specific effect involved, it is in the direction of further accelerating the formation of connective tissue, which is already being formed at a rapid rate at this period, and of increasing the fluid content of these tissues.

9. Donaldson, H. H.: A Comparison of the White Rat with Man in Respect to Growth of the Entire Body, Boas Anniversary Volume, New York, G. E. Stechert & Company, 1906.

It seems apparent from these and earlier studies that the eruption and growth of the teeth and of their investing tissues may be considered only as a part of the general phenomena of growth of the body, and may be influenced by the pituitary gland in a specific direction only insofar as the time and velocity of development of the various tissues and organs may be influenced.

THE ENDOCARDITIC PROCESS IN CHILDHOOD*

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The rarity of endocarditis during the first years of life has been remarked on by various writers in the past. Thus, Holt found no case of endocarditis in the course of 1,000 autopsies on infants under 3 years of age, and Hochsinger confirmed the extreme rarity of the condition in persons less than 5 years of age, as compared with its great frequency in those between the ages of 10 and 14. On the other hand, clinicians claim a relatively high percentage for valvular lesions occurring during the first ten years of life. Thus Guttmann (1893) reported that 5 per cent of cases of valvular disease occurred during the first ten years, 18 per cent between 10 and 18 years of age, and from 22 to 23 per cent after 20 years of age. More precisely G. Schultze (1906), working on a vast material, obtained the following data: 4.29 per cent of cases occurred from birth to 10 years, 36.60 per cent (the maximum percentage) from 10 to 20 years and 28.27 per cent from 20 to 30 years. After 30 years of age, the percentage becomes steadily lower.[1]

From these statistics it appears that valvular lesions occur with some frequency during the first years of life, referable in part at least to an unrecognized endocarditic process.

INCIDENCE OF VALVULAR ENDOCARDITIS IN CHILDREN, AS NOTED IN AUTOPSY REPORTS

I have tried to determine, as exactly as possible, the incidence of valvular endocarditis in children, and have made use of the postmortem material of the Institute of Pathological Anatomy of Florence, amounting to 4,952 autopsies. The statistics of age and sex are shown in figure 1. In all, 179 cases of endocarditis were noted, 93 of which were acute (verrucose, vegetative and ulcerative) and 83 subacute or recurrent. Figure 2 shows the relative frequency of the condition at different age periods and of itself is a demonstration of the rarity of anatomic evidences of endocarditis in childhood. The rarity becomes

* Submitted for publication, Dec. 5, 1930.

1. Jores, A., in Brüning, H., and Schwalbe, E.: Handbuch der allgemeinen Pathologie und pathologischen Anatomie des Kindesalters, München, J. F. Bergmann, 1921.

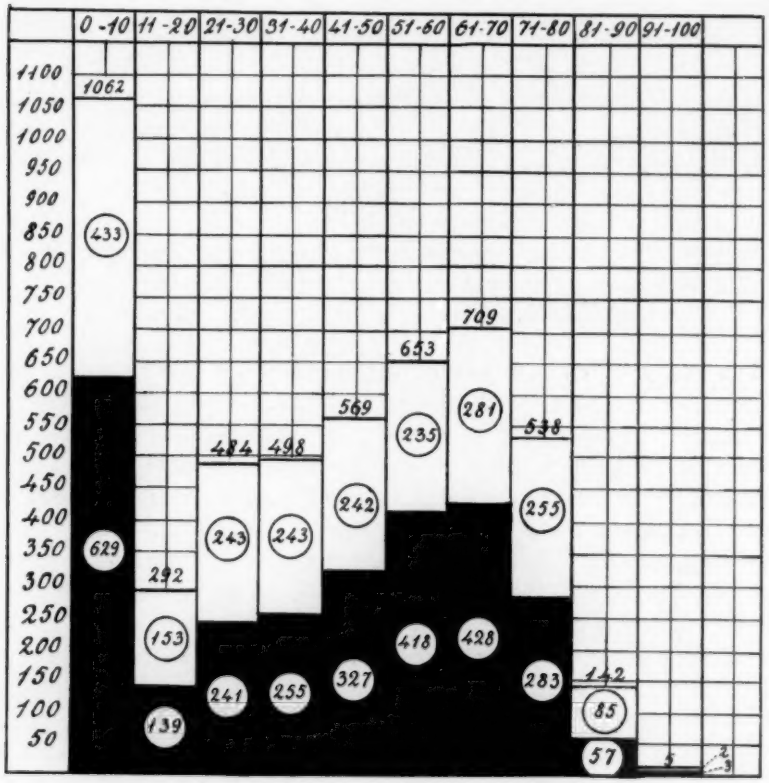

Fig. 1.—Distribution of the autopsy material according to age and sex. The solid black columns represent the male sex; the white columns, the female sex.

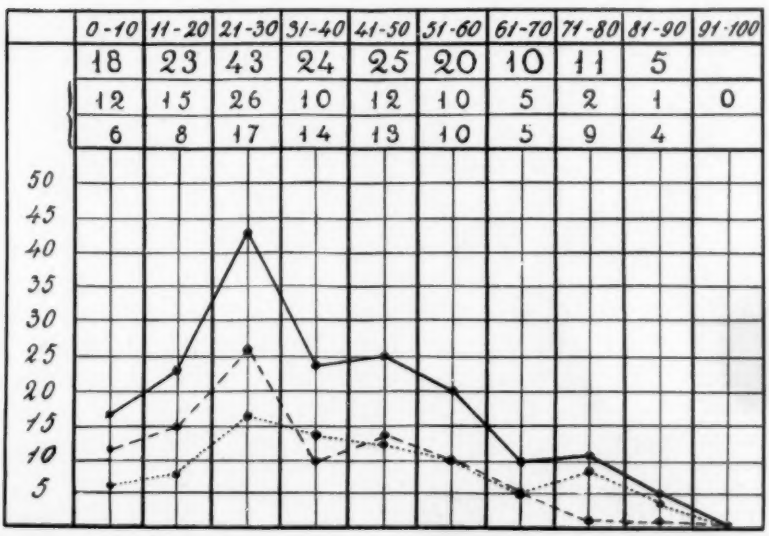

Fig. 2.—Distribution of the cases of endocarditis by age periods. The continuous line represents all the cases; the broken line, the acute cases, and the dotted line, the chronic or recurrent cases.

still more striking when the figures are reduced to percentages of the total autopsies in each age period, as in figure 3.

It is clear that during the first ten years of life valvular lesions of the endocardium are rarely found (18 cases among the 1,062 autopsies of that age period, i. e., 1.69 per cent). This rarity is still more striking for the first year of life, for of the 18 cases in the first decennium, only 2 occurred in infants under 1 year of age, 6 in children from 1 to 5, and 10 in children from 6 to 10 years of age.

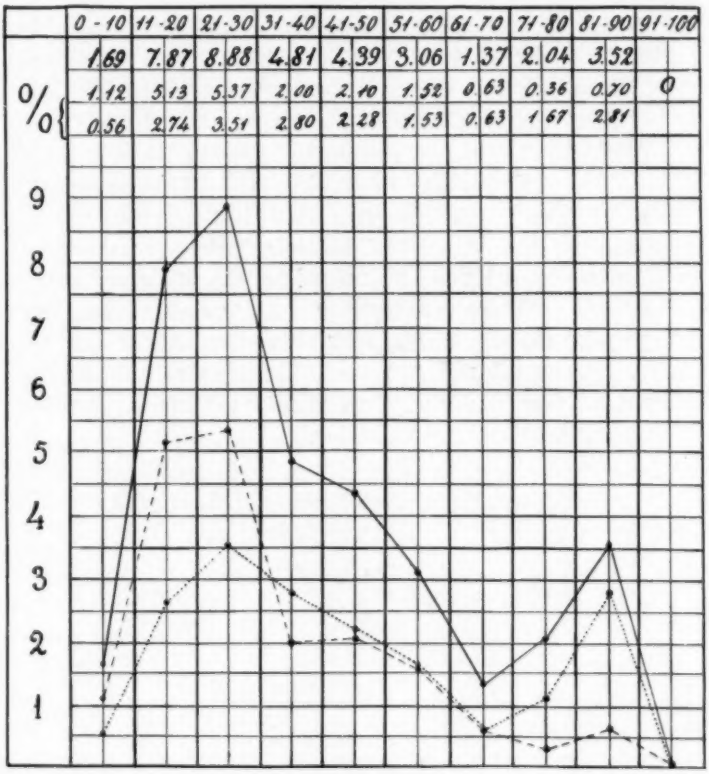

Fig. 3.—Percentage of cases of endocarditis in the total number of autopsies of each age period.

Interpretation of Apparent Infrequency.—In explanation of this rarity of valvular disease in infancy, Finkelstein assumed a special immunity of the endocardium of sucklings against inflammatory agents, severe and numerous though such agents are in this period. The real explanation is probably much simpler. The postmortem diagnosis of endocarditis, both in children and in adults, is usually based on macroscopic evidence of thrombotic vegetations on the valves or on the parietal endocardium. Macroscopic evidence of this kind is, however, cer-

tainly incomplete and fallacious. In persons dying of toxic infectious diseases, valves appearing perfectly healthy to the naked eye show microscopic changes, which are evidently inflammatory, even without the presence of any thrombotic deposits (Baldasjari,[2] de Vecchi,[3] Czirer [4]).

The diagnosis of endocarditis is therefore not always possible without a histologic examination, and ought to be extended to all cases in which any active participation of the valvular tissues can be demonstrated microscopically. Moreover, as I have maintained elsewhere,[3] the valvular changes are to be regarded as the characteristic feature of the process, while the thrombotic lesions must be considered as merely secondary changes, often absent throughout the course of the disease. The necessity of a histologic basis for the diagnosis of incipient valvular lesions, which I have long maintained, has received very tardy acknowledgment. Only recently Holsti [5] took up the idea and confirmed the great value of microscopic research in the diagnosis and interpretation of endocardial lesions that would otherwise escape detection.

These general considerations regarding the anatomic diagnosis of endocarditis are still further justified in the case of endocarditis in infants, in whom the valvular apparatus is more delicate and the initial changes in it more difficult to detect. Moreover, it is probable that before the third year of life (as in the embryo) the constitution of the blood (morphologically an erythropenia, chemically a deficiency of globulin) does not lend itself to the formation of thrombi. Hence there is an insufficiency of the elements necessary for the formation of endocardial vegetations.

MATERIAL USED FOR MICROSCOPIC STUDY

The present investigations have been directed toward a systematic study of these initial stages of endocarditis, as demonstrable histologically even when macroscopic examination does not suggest their presence.

The valves of the infant's heart lend themselves readily to such a study of the early phases. One is dealing with conditions almost experimental, because the disease is developing in tissues not previously altered. Naturally, the lesions in such cases are not widespread or marked. If they were, they would be evident to the naked eye. One deals always with incipient and circumscribed lesions; hence this material is useful in the interpretation of the pathogenesis of processes

2. Baldasjari: Tesi di Laurea, Bologna, 1907; Centralbl. f. allg. Path. u. path. Anat. **20**:97, 1909.

3. de Vecchi, B.: Arch. di pat. e clin. med. **4**:3, 1926.

4. Czirer, L.: Virchows Arch. f. path. Anat. **213**:272, 1913.

5. Holsti, O.: Arb. a. d. path. Inst. zu Helsingfors **51**:401, 1928.

Cases Examined

Case	Autopsy	Age	Sex	Anatomic Diagnosis
1	16523	1 mo.	F	Intestinal malformations; volvulus; laparotomy, purulent peritonitis
2	301;N.S.	2 mo.	M	Tuberculous mastoiditis; miliary tuberculosis; meningitis
3	15064	3 mo.	M	Status thymolymphaticus; confluent bilateral bronchopneumonia
4	14718	3 mo.	F	Suppurative cerebrospinal leptomeningitis; abscess of brain
5	15054	4 mo.	M	Hyperplasia of Peyer's patches; multiple subcutaneous abscesses
6	15046	4 mo.	F	Diphtheritic laryngitis; bilateral bronchopneumonia
7	16429	6 mo.	F	Bilateral confluent bronchopneumonia
8	16428	7 mo.	M	Bronchopneumonia on right side
9	16496	7 mo.	F	Metapneumonic empyema on left side
10	15045	8 mo.	M	Bilateral bronchopneumonia; hemorrhagic pulmonary infarcts; softening; purulent pleurisy on left side; parenchymatous degeneration
11	14762	8 mo.	M	Bilateral bronchopneumonia; empyema on right side; acute nephritis; splenic tumor
12	14820	8 mo.	M	Suppurative leptomeningitis of vault
13	14763	9 mo.	M	Bilateral bronchopneumonia; rickets; hypoplasia of right kidney; parenchymatous degeneration
14	16432	9 mo.	F	Bronchopneumonia on right side
15	16074	10 mo.	M	Acute enteritis; nasal impetigo; parenchymatous degeneration
16	16420	12 mo.	M	Bilateral bronchopneumonia
17	14554	12 mo.	M	Hyperplasia of Peyer's patches; splenic tumor; mesenteric lymphadenitis; bilateral pleurisy; serofibrinous pericarditis; septic infarcts of lung; acute nephritis
18	16416	13 mo.	M	Bilateral bronchopneumonia; pericarditis; bilateral fibrinous pleurisy
19	16418	13 mo.	M	Acinonodose tuberculosis of both lungs; nodular tuberculosis of spleen and liver
20	180;N.S.	14 mo.	M	Epidemic cerebrospinal leptomeningitis
21	16519	16 mo.	M	Bilateral bronchopneumonia; thrombosis of suprahepatic vein; ascites; splenomegaly
22	14819	16 mo.	M	Bilateral bronchopneumonia; bilateral fibrinous pleurisy; suppurative leptomeningitis
23	16732	20 mo.	M	Typhoid enteritis; suppurative otitis media on left side
24	16755	21 mo.	F	Old tonsilar phlegmon; bilateral bronchopneumonia; chronic mitral endocarditis; multiple cerebral softening; acute cholecystitis; splenomegaly
25	16417	2 yr.	F	Bilateral bronchopneumonia; pertussis
26	16412	2 yr.	M	Bilateral bronchopneumonia; suppurative pleurisy on right side; pleurectomy
27	16502	2 yr.	F	Unresolved pneumonia; bilateral suppurative pleurisy; parietal cardiac thrombosis
28	15074	3½ yr.	F	Postdiphtheritic paralysis; bilateral bronchopneumonia; endocarditis verrucosa mitralica acuta
29	16734	3½ yr.	F	Necrotic angina; septicemia; macular facial erythema
30	14680	3½ yr.	F	Bronchopneumonia on right side; status thymolymphaticus
31	16426	4½ yr.	M	Osteomyelitis of right tibia; fibrinous pericarditis; multiple pulmonary infarcts
32	16486	5 yr.	M	Acute pharyngolaryngotracheal inflammation; suppurative otitis media
33	16733	5 yr.	M	Necrotic scarlatinal angina; bed sores; acute nephritis
34	14654	5 yr.	M	Descending diphtheria; follicular enteritis; induration of the aortic margin of the mitral valve
35	16360	5 yr.	F	Descending diphtheria; acute bronchitis; focal pulmonary atelectasis; meningo-encephalic congestion
36	16507	6 yr.	M	Purulent arthritis of left knee; empyema on right side; multiple pulmonary infarcts
37	16430	7 yr.	F	Mastoidectomy on right side; acute serous leptomeningitis
38	16147	7 yr.	F	Suppurative osteomyelitis of petrous portion of temporal bone; multiple pulmonary abscesses; hematogenous suppurative nephritis; verrucose endocarditis of mitral valve
39	16431	8 yr.	M	Necrotic appendicitis; localized fibrinous peritonitis; chronic pulmonary tuberculosis
40	141;M.S.	9 yr.	M	Measles; bronchopneumonia on right side
41	16490	14 yr.	M	Bilateral bronchopneumonia; diplococcal sepsis
42	16491	16 yr.	F	Bilateral pulmonary tuberculosis (ulcerocaseous)

that usually are studied only at a much more advanced, more complex stage.

The material that I have used for my own observations comprises forty-two cases, selected from autopsies performed during the past few years. I have not considered it necessary to extend these observations over a longer period, because, as will be seen, the results are sufficiently clear and consistent.

As regards valves presumably affected, particularly as regards experimental results,[6] I must refer to considerations set forth elsewhere, which explain the difficulty of basing the observations on a large amount of material. It is essential that the suspected valve be subjected to thorough examination, and not to a superficial, hurried search. Valves that when first seen under the microscope appear unaffected show obvious lesions in other sections. In order to make an exact diagnosis, it is therefore necessary to search through the whole valvular apparatus, sometimes in serial sections, under a suitable magnification, for only so is it possible to detect lesions that might otherwise escape notice. All this requires system, time and patience; therefore, the cases studied cannot be very numerous.

From the anatomic diagnosis it is seen that only four cases (24, 28, 34 and 38) showed macroscopic changes in the valves. In two of these (28 and 38) there were the lesions of recent verrucose endocarditis; in the other two (24 and 34) the lesions were those of chronic sclerosing endocarditis. I might have included other cases in the list, as occurring in infancy, but have restricted myself to these four cases for comparison with those in which there was no visible lesion in the valves.

The material is subdivided into eight groups on a nosologic basis. This grouping proves useful for the demonstration of the histologic results.

HISTOLOGIC OBSERVATIONS

Group 1.—Group 1 included five cases (5, 23, 31, 36 and 38) of suppurative processes in various parts of the body (osteomyelitis, arthritis, otitis complicating typhoid fever, cutaneous abscesses, etc.), in which there was very probably a septic process at work.

The valves of the heart in these cases always showed histologic lesions, varying in severity and extent. As would be expected, the most intense lesions occurred in case 38, in which the valvular lesions were already visible macroscopically on the mitral segments. In an extensive area on the atrial surface of the mitral valve, there was loss of endothelium with hyalinization and necrosis of the adjoining valvular tissue. The desquamated endothelium was largely enclosed in a

6. de Vecchi, B.: Le malattie del cuore, Rome, 1923, vol. 7, p. 72.

fibrinous network. There were numerous cells of lymphocytic type in the deeper layers of the valve, surrounding the regressive lesions in the valvular tissues. In the subendothelial layers nearest the desquamated area there were large cells, each with abundant cytoplasm and a vesicular nucleus. These were easily interpretable as histiocytes (fig. 4 *A*). In other parts of the valve, even where the endothelium was apparently healthy, evidences of histiocytic reaction were still to be met with, and this not only in the superficial subendocardial layers, but also extending into the deepest layers of the valvular tissue, and sometimes accompanied by an abundant and diffuse lymphocytic infiltration. Evidence of hyaline thrombosis following the desquamation of endothelium was found in some sections. In some parts one might also see deposits of fibrin, enmeshing erythroctyes and leukocytes. A migration of elements originating in the underlying tissues into the initial thrombus could not, however, be observed. The thrombus was a passive phenomenon occurring subsequent to the formation of the lesions established on the valve.

That lesions of this type were present in areas macroscopically healthy was proved by the fact that a histologic examination of the apparently healthy aortic valve in the same case showed areas of endothelial desquamation and edema with detachment of the underlying valvular layers, a somewhat diffuse infiltration by lymphocytes and the presence of histiocytic elements both in the superficial and in the deeper layers. The type of lesion was the same; the difference was in the intensity of the pathologic change and above all in the absence of the secondary phenomenon of superficial thrombosis.

Similar phenomena were evident in the other cases also: limited endothelial desquamation, edema and hyalinization of the valvular layers, lymphocytic infiltration and histiocytic reaction. The latter was marked, especially at the points of attachment of the valves, and was not necessarily accompanied by desquamation of the endothelium. In case 23 (purulent otitis complicating typhoid enteritis) the lesions were marked and particularly extensive, the histiocytic reaction extending down into layers far distant from the endothelial lesion.

Group 2.—Group 2 included four cases: one following scarlet fever (33); two of acute type, and followed probably by septicemia (29 and 32), and the last (24) with healed anginal lesions, but with bronchopneumonia, splenomegaly and septicemia, thickening of the edges with thrombotic deposits on the mitral valve, and, as a consequence of the detachment of the latter, the formation of multiple foci of cerebral softening.

As was to be expected, histologic examination in case 24 showed very serious lesions on the mitral valve. These consisted in an intense, widely spread edema, with hyalinization of the valvular layers, accom-

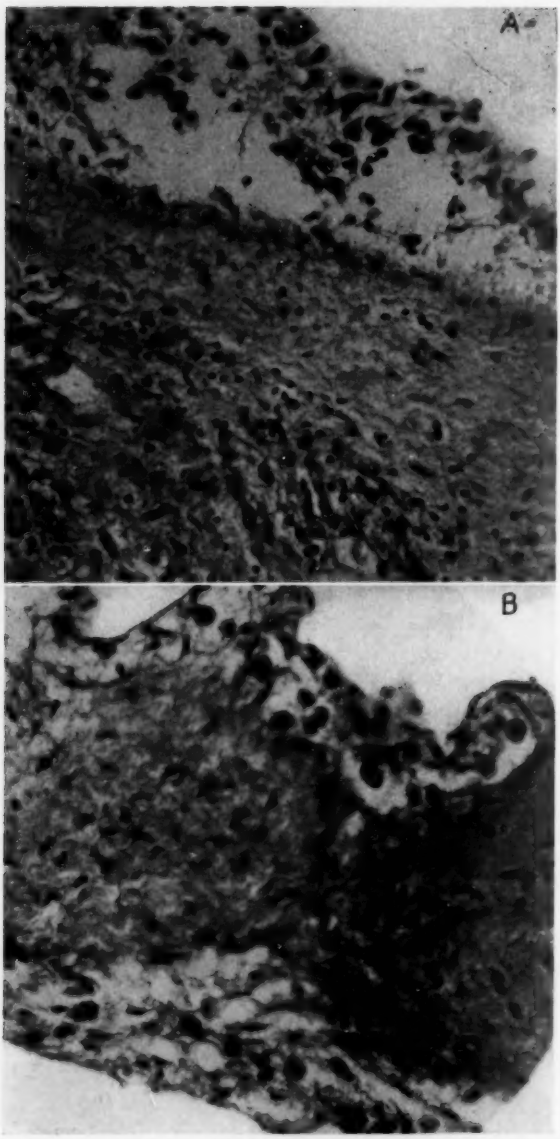

Fig. 4.—*A*, mitral valve in case 38, that of a boy, aged 7, with suppurative osteomyelitis in petrous bone, abscesses of the lung, suppurative nephritis and verrucose mitral endocarditis. The initial stage of the latter was visible macroscopically. The photomicrograph shows desquamation of endothelium and agglutination of the exfoliated cells in a fibrinous network, hyalinization and partial necrosis of the valvular tissue, and lymphocytic infiltration and deep histiocytic reaction delimiting the degenerative necrotic zone.

B, Aortic valve in case 24, that of a girl, aged 21 months, with tonsillar phlegmon, cholecystitis, multiple cerebral softening and verrucose endocarditis of the mitral valve. The latter was visible macroscopically. The aortic valve was macroscopically intact. The photomicrograph shows desquamation of the endothelium, edema and histiocytic reaction of both surfaces of the valve.

panied by an intense histiocytic reaction, which passed into the deeper structure, but was less marked near the surface. In the same case identical and equally extensive lesions were seen on both the ventricular and the arterial surfaces of the aortic valve, although this appeared normal to the naked eye (fig. 4 *B*).

A marked superficial and deep histiocytic reaction, accompanied by a less marked lymphocytic infiltration, appeared in case 29, both in the mitral and in the aortic valve. A typical focus of endocarditis, with extensive desquamation of the endothelium and agglutination thereof in a fibrinous network, with intense lymphocytic infiltration and peripheral histiocytic reaction, had developed in the aortic valve in case 32 (fig. 5 *A*); in this case, too, the valves appeared normal to the naked eye. Valvular lesions, but much milder and more circumscribed, were also present in a case of scarlatinal angina (33).

Group 3.—Four cases of diphtheritic infection were studied. Of these, three were of acute type (6, 34 and 35), and one (28) had run a more chronic course, death being due to postdiphtheritic paralysis. In case 28 the autopsy showed acute verrucose endocarditis of the mitral valve, while in case 34 there was evidence of induration of the aortic segment of the mitral valve.

The tissues obtained in case 28 were insufficiently fixed, so that a proper study of them could not be made. In case 34, lesions of the infiltrative and histiocytic reaction type were present not only in the mitral valve, but also in the aortic (fig. 5 *B*), though the latter was macroscopically normal.

In case 6 (diphtheria in a suckling) the mitral valve showed a few foci of lymphocytic infiltration and histiocytic reaction in the valvular layers, with partial desquamation of the endothelium. But of special importance were the histologic lesions in case 35, in which the valves had appeared healthy at the autotpsy. Both the mitral and the aortic valves showed areas of endothelial desquamation, with extensive hyalinization and incipient necrosis of the underlying valvular layers, also slight lymphocytic infiltration and histiocytic reaction, not only in the superficial subendothelial structure, but also in the deep layers, especially in the border area between the degenerative necrotic, and the healthy, valvular tissue (fig. 6 *A*).

Group 4.—There were four cases of suppurative leptomeningitis, two being probably primary epidemic (4 and 20) and two secondary (12 and 37). Focal lesions were demonstrated histologically in the valves in three cases, especially in the mitral valve in case 12 (edema of the valvular layers, deep histiocytic reaction). Only in case 37 did the valves show no histologic lesion.

Group 5.—Group 5 included two cases of acute exudative peritonitis: one (1) in a new-born infant, secondary to operative interference, and the other (39) in a child with tuberculosis following necrotic appendicitis. Infiltrative and reactive lesions of the valvular

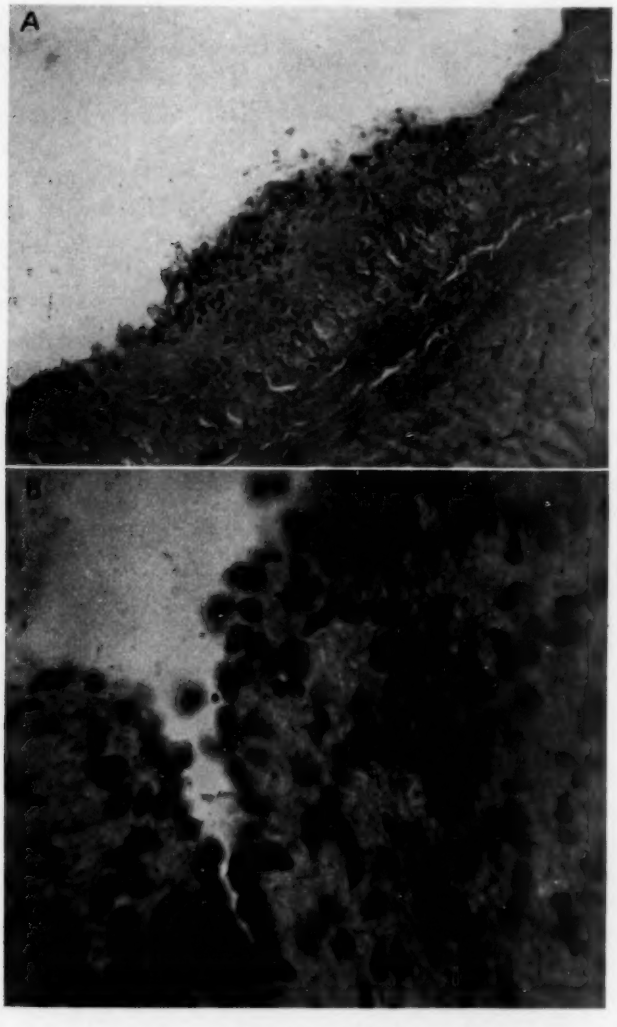

Fig. 5.—*A*, aortic valve in case 32, that of a boy, aged 5, with acute pharyngolaryngitis and suppurative otitis. The valve was macroscopically healthy. A typical focus of commencing endocarditis is shown.

B, aortic valve in case 34, that of a boy, aged 5, who died from descending diphtheria. Thickening of the mitral valve was found. The aortic valve was macroscopically normal. Note lymphocytic infiltration and diffuse deep histiocytic reaction.

tissue were found in both cases, but they were of particular intensity in case 1, in which the infiltrative and histiocytic reactions were most marked in the deeper layers of the valve; that is, at a point distant from its free margins.

Group 6.—Two cases of enteritis comprised group 6: one was acute (15); the other was more chronic and complicated by pleuropericarditis

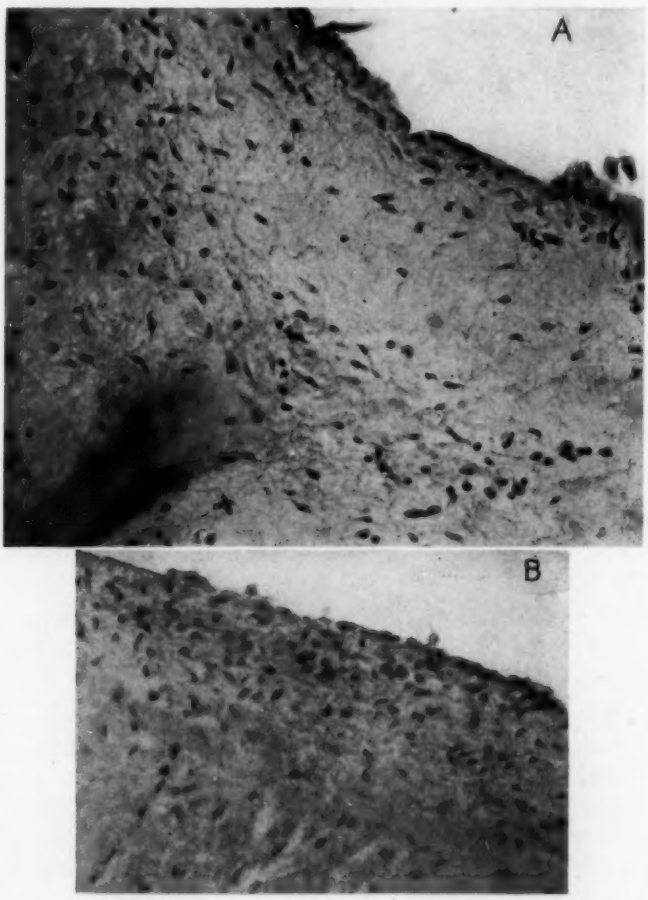

Fig. 6.—*A*, mitral valve in case 35, that of a girl, 5 years old, who died from diphtheria. The valve was macroscopically normal. Note shedding of endothelium, hyalinization and necrosis of endothelial layers, lymphocytic infiltration and subendothelial and deep histiocytic reaction.

B, mitral valve in case 40, that of a boy, aged 9, who died from measles complicated by bronchopneumonia. Note marked lymphocytic infiltration and histiocytic reaction in the valvular layers.

and secondary infarction of the lung (17). The lesions were slight in both cases, especially in case 17.

Group 7.—In group 7 were included a number of cases of bronchopneumonia (eighteen cases), of which twelve occurred in infants under 2 years of age and six in children above this age.

Of the cases in suckling infants, seven were of fatal bronchopneumonia uncomplicated by other diseases (3, 7, 8, 13, 14, 16 and 21). In some of these cases the valves showed no obvious lesions; in others (13 and 14), there was partial desquamation of endothelium with homogenization of the valvular layers and lymphocytic infiltration, with diffuse, deep-seated histiocytic reaction. In five cases of bronchopneumonia in infants (9, 10, 00, 18 and 21), in which various complications were present (empyema, fibrinous pleurisy, pulmonary infarction with septic softening, pericarditis, meningitis, etc.), the valvular lesions did not appear more serious or widespread than in the first-mentioned cases; here, also, besides some cases in which the valves gave no indication of lesions (9 and 10), there were others (as case 11) in which the valves showed signs of inflammatory reaction.

In six cases of bronchopneumonia in children over 2 years of age (25, 26, 27, 30, 40 and 41) marked lesions were not met with, even in cases with serious complications, in which one would have expected to find particularly severe lesions (27, pneumonia without resolution; 41, sepsis of diplococcal (?) origin; 26, pleurisy and pericarditis). Marked lesions were present only in one case of bronchopneumonia secondary to measles (40); here there was the usual type of lymphocytic infiltration and histiocytic reaction in the valvular layers (fig. 6 *B*).

Group 8.—Three cases (2, 19 and 42) of various forms of tuberculosis in children of different ages comprised group 8. There were scattered lymphocytic infiltrations in the aortic valve in case 42 (ulcerocaseous pulmonary tuberculosis); on the other hand, these were particularly well marked in case 2 (tuberculous mastoiditis, miliary tuberculosis, tuberculous meningitis), in which the aortic valve especially showed evident signs of endothelial desquamation with an intense lymphocytic and histiocytic reaction. The lesions were still more marked and diffuse in case 19 (acinonodular pulmonary tuberculosis with nodular tuberculosis of the liver and spleen), in which both the mitral (fig. 7 *A*) and the aortic (fig. 7 *B*) valves were involved in an extensive endothelial desquamation, with intense lymphocytic infiltration and a diffuse histiocytic reaction in the superficial, as well as in the deep, layers of the valve tissue.

In the valvular tissues of infants under 12 months of age dying of diseases of toxic infective type, without demonstrable evidence of sepsis, there were often histologic changes that could not be demonstrated or

suspected macroscopically. These lesions had similar characteristics, and they differed from one another mainly in their distribution and intensity; usually they were confined to a few areas along the valvular margins, so that only an accurate histologic examination revealed them.

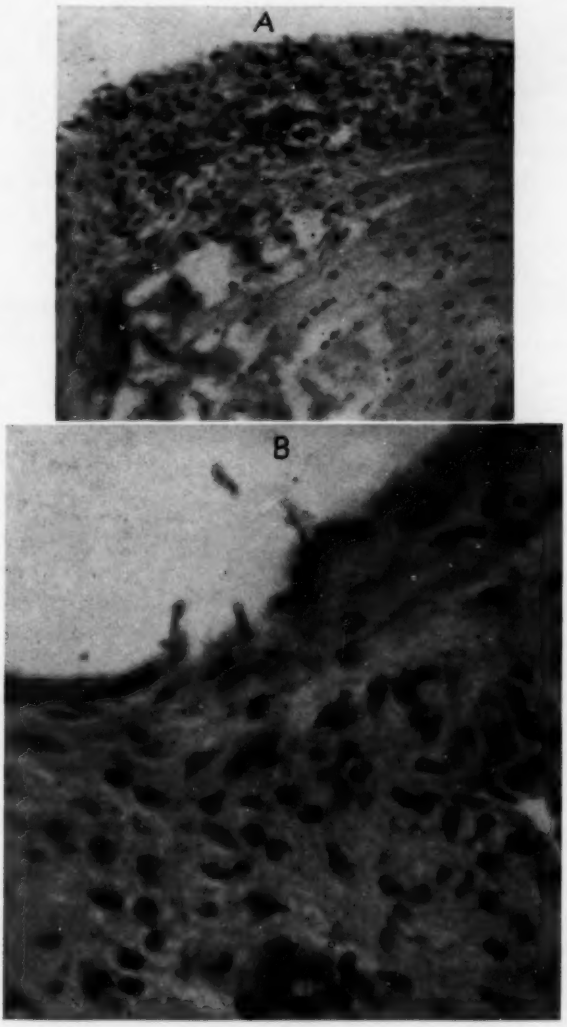

Fig. 7.—*A*, mitral valve in case 19, that of a boy, aged 13 months, who died from acinonodular tuberculosis of the lungs and nodular tuberculosis of the spleen and liver. The photomicrograph shows desquamation of the endothelium, lymphocytic infiltration and deep-seated, diffuse histiocytic reaction.

B, aortic valve, showing desquamation of the endothelium, edema, lymphocytic infiltration of the subjacent valvular layers and intense histiocytic reaction.

It often happened that many sections of a valve had to be searched through before a single focus of disease could be met with. Usually the affected areas were situated near the free margins of the valves, where the mechanical effect of the jolting together of the edges of the valves rendered them more susceptible to an inflammatory stimulus; only exceptionally and in the severer cases were the lesions more widely distributed over the valvular tissues.

The lesions consisted in swelling and subsequent desquamation of the endothelium (very rarely, however, it underwent proliferation); edema followed by hyalinization of the underlying connective tissue of the valve, with formation, in some cases, of limited foci of necrosis; lymphocytic infiltration, sometimes in the superficial, but often in the deep, layers, generally on the borderline between the still healthy tissue and the foci of necrobiosis, and finally, active histiocytic reaction corresponding to the lymphocytic infiltration, hence sometimes in the superficial subendothelial layers and sometimes extending also to the deeper layers. Rather exceptionally, there was present a scanty number of polymorphonuclear leukocytes; these occurred only in very severe and relatively advanced cases. Early thrombotic phenomena overlying the valvular lesions were rarely demonstrable.

PATHOGENESIS OF LESIONS

The interpretation of the microscopic changes is unequivocal. The changes in the valvular tissues are partly passive (degenerative, necrotic) and partly active (inflammatory). These processes are superimposed and intermingled. One deals with a primary focal valvulitis.

I use the term "valvulitis" instead of "endocarditis" because that term better expresses the nature of the lesions. The word "endocarditis" refers to a more complex process and applies rather to the macroscopic aspect of the lesions produced by the presence of thrombotic vegetations. Hence several authors, following the original researches of Ziegler, insisted rather on the term "thrombo-endocarditis," as if the two factors included in the one term are necessarily connected. So much credence have pathologists given to this that Roesner [7] even considered valvular thrombosis and verrucose endocarditis to be one and the same condition, and more recently Krischner,[8] following the ideas advanced by Beitzke, proposed to classify endocarditis according to the macroscopic appearance and the character of the valvular thrombosis.

This way of regarding endocarditis I have long thought to be objectionable,[3] and the observations here set forth confirm me in this opinion. The formation of the thrombus is a secondary phenomenon which may

7. Roesner, E.: Klin. Wchnschr. **3**:297, 1924.
8. Krischner, H.: Virchows Arch. f. path. Anat. **265**:545, 1927.

be entirely absent; hence it cannot be taken as an essential feature of the process. The error of interpretation on the part of authors who have studied the essential nature of the endocarditic process in man has arisen from their observing cases which were apparently in the initial stage, but which actually were far enough advanced to be complicated, and marked, as it were, by the secondary formation of thrombus.

Nor do I believe it correct to accept the views proposed by Jegoroff.[9] He considered septic endocarditis to be the only true form and supposed (but did not prove) this process to be always set up by septic emboli in the blood vessels of the valves, while admitting that these may be in a perfectly normal condition. The milder cases, of the verrucose type, the endocarditis simplex of other authors, which is generally nonmicrobic (endocarditis toxica), should be considered, according to Jegoroff, not as endocarditis, but as endocardosis, and is caused, not by vascular emboli in the valves, but by toxic agents acting directly on the valvular endocardium.

Leaving aside the serious objections to Jegoroff's pathogenic mechanism of septic endocarditis, I think that the new term "endocardosis" is not acceptable, because it suggests only essentially passive processes in the tissues, whereas one actually has to do in these cases with active processes, characterized by lymphocytic infiltration and histiocytic reaction; that is, processes truly inflammatory. The term "endocarditis," or better, "valvulitis," is therefore to be applied to these conditions because it exactly expresses the nature of the process.

Dietrich,[10] on the other hand, interpreted the process of endocarditis on a reactive inflammatory basis (in the modern sense of the word), and worked out the conception in a series of experiments, following ideas already expressed by Rosenow[11] (namely, that the course and evolution of the infection are due more to a process of bacterial immunization against the antibodies of the host, and so, ultimately, to the original virulence of the germ). These ideas have been adopted by me to explain the pathogenesis of endocarditis,[3] and also by Siegmund[12] in some of his original experiments. Dietrich, producing general infections in animals by vaccination, obtained the phenomena of valvular endocarditis, which he considered to be produced by circulating organisms lodging on existing foci of reaction in the endothelium. The final form of the lesion varies with the modifications

9. Jegoroff, B.: Arch. d. mal. du coeur **19**:647, 1926; Ztschr. f. klin. Med. **103**:584, 1926.

10. Dietrich, A.: Ztschr. f. d. ges. exper. Med. **50**:85, 1926; München. med. Wchnschr. **75**:1328, 1928.

11. Rosenow, E. C.: Immunological and Experimental Studies on Endocarditis, J. Infect. Dis. **6**:245, 1909; **7**:411 and 429, 1910.

12. Siegmund, H.: Centralbl. f. allg. Path. u. path. Anat. **36**:226, 1925.

induced in the organisms by contact with the tissues, that is, with the condition of local immunity in the tissues; hence in the valvular tissues the fate of the process depends on the power of reaction and reabsorption. If these tissues succeed in overpowering the noxious action of the organisms, lesions of the vegetative type result, followed by proliferation of the valvular tissue; but if, on the contrary, the tissues are overpowered, a widespread valvular necrosis occurs, followed by thrombotic deposits and lesions of an ulcerative and polypoid type. Relapses are provoked by alternating changes in the reaction of the tissues. Even endocarditis lenta is not characterized by a strict specificity of organism (*Streptococcus viridans*), but by changes in the mutable endothelial reaction occurring during the course of a chronic and general infection of streptococcic type.

Dietrich's ideas certainly seem acceptable. They confirm and renew the interpretation of endocarditis as inflammatory, putting it in relation with the powers of regulation and defense of the whole organism; and they bring the subject of endocarditis into line with modern views on the pathogenesis of infectious diseases, in general, and of the tuberculous process, in particular.

Some of the points, however, are debatable: First of all, some important experiments by Semsroth and Koch [13] (1929) tended to prove that in the localization of bacteria causing endocarditis, the allergic sensitization preceding the general infection might not be specific, but might be provoked by other causes, such as foreign, nonbacterial protein. Similarly, Klinge [14] obtained sensitization of rabbits toward the rheumatic virus, by using injections of horse serum. Hence, the nodular, degenerative proliferations met with in animals, resembling those found in persons with articular rheumatism (Aschoff bodies), should be considered as a mesenchymal, and not a specific, reaction to the particular organism. These important observations greatly widen one's conception of allergy as a preliminary factor in valvular lesions.

Furthermore, even if the allergic factor has a certain importance in the production of the endocardial lesions, one must not underrate the importance of the elective attraction of a micro-organism and its toxic products toward the valvular tissues. This factor of special attraction is well established by experimental work [15] and occurs in spontaneous disease; [16] there is a tendency for certain types of organism (as compared with others otherwise apparently similar) to give rise to endocarditic lesions.

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13. Semsroth, K., and Koch, R.: Studies on the Pathogenesis of Bacterial Endocarditis, Arch. Path. **8**:921, 1929.

14. Klinge, F.: Beitr. z. path. Anat. u. z. allg. Path. **83**:185, 1929.

15. de Vecchi, B.: Boll. sc. med. **8**:15, 1908.

16. Haden, Russel: J. Lab. & Clin. Med. **12**:31, 1926.

Another point may be referred to in regard to Dietrich's conceptions, namely, the presence and import of an endothelial reaction. At least in the initial stages of valvulitis, such as are here described, the endothelium shows no reaction. It only desquamates and disappears, leaving the underlying layers bare. The reaction takes place only in the connective tissue of the valve, with a resultant lymphocytic infiltration and the formation of large elements of epithelioid type, which are to be interpreted as the histiocytes of a reticulo-endothelial reaction, varying in different cases and according to the time the lesions have taken to develop.

A histiocytic reaction in the valvular tissue was noted, though not adequately interpreted, by Koeniger in his classic contribution to the histology of rheumatic endocarditis. This reaction has been confirmed by more recent observations (Darre and Albot [17]), and it has been noticed that in rheumatism and endocarditis lenta an endothelial (or better, "mesenchymal") reaction develops in various parts of the organism (Merklen and Wolff,[18] Istamanowa [19]). This makes the interpretation of the Aschoff bodies in spontaneous and experimental disease clear.[15] Huguenin and Albot [20] demonstrated deep histiocytic reactions in valvular tissues in cases of subacute endocarditis, similar to those met with in the rheumatic forms.

The present observations on the initial stages of acute endocarditis developing in the valves in children as a result of various causal agents confirm these conceptions. The inflammatory reactions show, first, a definite activity of the mesenchyma. Then there is lymphocytic infiltration, and histiocytes appear in the connective tissue of the valves even in areas distant from the superficial layers. The inflammatory reaction in the initial stages of the process is always of the same type, whatever the stimulus. In the later stages, the course and evolution of the process vary according to the nature of the micro-organisms and the resistance of the subject.

ETIOLOGY OF THE LESIONS

The importance of the part played by micro-organisms in the development of endocarditis is shown by the fact that in recent years many attempts have been made to arrive at a bacteriologic classification; this conception is not new, but the progress of technic has made it more feasible. For instance, Cowan,[21] in a recent research, classified acute endocarditis as produced by an anhemolytic streptococcus (rheumatism)

17. Darre, H., and Albot, G.: Ann. d'anat. path. **6**:465, 1929.
18. Merklen, P., and Wolff, M.: Presse méd. **36**:97, 1928.
19. Istamanowa, T.: Virchows Arch. f. path. Anat. **268**:224, 1928.
20. Huguenin, R., and Albot, G.: Ann. d. path. Anat. **7**:490, 1930.
21. Cowan, J.: Glasgow M. J. **108**:249, 1927.

and by the influenza bacillus. This classification is certainly neither complete nor exact, but would be useful if it could be extended to all observed cases. It would be easier to do this in the clinic than in the autopsy room, where bacteriologic researches are more uncertain and difficult. It is for this reason that, in the present research, save in a few isolated cases, no systematic bacteriologic examinations were made, and so a bacteriologic classification of the cases of incipient endocarditis cannot be attempted. A search for micro-organisms in these sections almost always yielded negative results, and the few positive results do not allow one to draw any useful conclusion. I am obliged to confine myself to a grouping of the cases according to the various types of disease, and this is, of course, an inexact and even fallacious criterion.

In some of the cases observed, especially in groups 1, 2, 4 and 5, it is probable that at a certain period of evolution of the disease, there was a more or less advanced condition of sepsis. In all the definitely grave cases, and in some others in which the valvular lesions were visible macroscopically, the genesis of the lesions is readily explained by the lodging of micro-organisms on the valves owing to some mechanical cause (reciprocal impacts of the valves, formation of whirlpools and eddies in the blood) or to congenital causes (congenital malformations), as has been recently suggested by Grant, Wood and Jones.[22] But acquired morbid changes and antecedent processes cannot be taken as etiologic factors in the case of involvements of the valves in infants and experimental animals.

In the great majority of cases, however, sepsis was not admissible, and the clinical course and anatomic observations do not leave any doubt about this. Even if the micro-organisms were, or had been, present in the circulation, they would have been few, certainly much fewer than in the mild and transitory bacteremia that occurs in almost all acute infectious diseases. It is logical, then, to admit that in all these cases the main factor was the deleterious effect on the valves of the toxic products of the germs (endocarditis toxica), the possibility of the existence of which is admitted by all pathologists, being proved by anatomic, as well as by experimental, observations.[23] With such a genetic hypothesis it is possible to explain the relative mildness of these lesions and the readiness with which they heal.

In the other groups of disease the cases of bronchopneumonia, diphtheria and tuberculosis call for consideration.

Bronchopneumonia.—In several cases of bronchopneumonia, both in sucklings and in children, whether uncomplicated or complicated by

22. Grant, R. T.; Wood, J. E., and Jones, T. D.: Heart **14**:247, 1928.

23. de Vecchi, B.: Arch. anat. pat. **1**:151, 1906. Ravenna, E.: Arch. per le sc. med. **44**:268, 1921.

some other condition, such as pleurisy, pericarditis, meningitis, etc., the valve was healthy to the naked eye, but showed microscopic changes. From these observations I must conclude that the action of the germ which caused the pneumonic process (usually the pneumococcus) was not limited to the pulmonary tissues, but extended to the cardiac valves. It was known that the pneumococcus has the power of acting on the cardiac valves (especially the aortic) and of originating endocarditis, usually vegetative, and of giving rise to grave lesions that usually appear toward the end of the disease or even during convalescence. But it was not known that the initial lesions are so frequently present in the valves during the course of pneumonic processes. It would be useful to ascertain whether similar lesions are also present in adults dying of pneumonia. It is not easy to establish the nature of this lesion; that is, whether it is due to the direct action of the circulating organisms on the valves (there is often a bacteremia in pneumonia), or whether it is a toxic action. The type of the lesions and their extent are in favor of the latter hypothesis.

Diphtheria.—As regards the diphtheritic process, the observations bring to light the frequency with which valvular inflammatory lesions occur in the course of this infection. The anatomic and experimental researches on the condition of the heart during diphtheria have been mainly directed toward the investigation of the state of the myocardium, only slight attention being paid to endocardial lesions arising during the course of this disease. The observations of Scagliosi, Fraenkel, Christoph, Howard and others showed that it is possible to find endocarditis of verrucose type in persons who have died of diphtheria. I do not know of really systematic researches on this point. My own experimental investigations [23] on the endocarditis provoked by bacterial toxins proved that the diphtheritic toxin is able to initiate localized inflammatory reactions in the valves in the rabbit, which are followed by the formation of secondary thrombi. These experiments were controlled and confirmed by Ravenna.[23] Systematic observations on the cadaver and some experiments go to prove that the valvular apparatus takes part in the processes induced by the Löffler bacillus, and that the relative rarity of thrombo-endocarditis in persons dying of diphtheria applies only to the most evident and advanced stages of a much commoner and more diffuse process. All the more logical is it to suppose a toxic genesis for this class of changes, as do all authors who wish to attempt an explanation, basing their opinions chiefly on experimental data.

Tuberculosis.—Of great interest are the marked changes found in the apparently healthy valves of children affected by tuberculosis.

Experiments (Michaelis and Blum,[24] Bernard and Salomon,[25] my own [26]) have confirmed the old anatomic and clinical observations that the bacillus of Koch can lodge on the valves and originate valvulitis. Michaelis and Blum agreed that it could cause specific histologic lesions (epithelioid and giant cells, foci of caseation)—a claim which should be received with the greatest reserve. But to explain the tuberculous forms of endocarditis that evolve without any possibility of the finding of the bacilli in the thrombus, or with the finding of the bacilli only in very small numbers, another view has been gaining credence—that in the genesis of these valvular lesions in the subjects of tuberculosis it is the toxic action, rather than the direct action, of the germ, in whatever way it comes in contact with the valves. This conception of the toxic origin of tuberculous endocarditis has been confirmed by my experiments, and Mencarelli [27] supported it in recent work in which he especially studied the action of the tuberculosis toxin on the endocardium.

General conclusions may also be drawn in reference to the various kinds of change and their frequency at different ages. From these observations it appears to be clear that in all persons with general toxic infectious diseases, the valves are more frequently and more severely damaged. The cases of endocarditis visible on examination with the naked eye which I have studied were all in children over 2 years of age. This does not apply to the microscopic lesions; very conspicuous and grave lesions have been found even in sucklings (cases 5, 6, 11, 13, 14, 19, etc.), and even in the first few days of life (1 and 2). This shows that at all ages, independent of previous lesions (i. e., the valves being perfectly normal), valvular lesions may develop to a notable intensity and extent, and the evolution of events varies with the circumstances that originate or accompany them.

EVOLUTION AND END-RESULTS OF THE LESIONS

All these valvular lesions are to be considered with reference not only to their genesis, but also with reference to the consequences that may follow later. One might suppose that lesions found on microscopic examination occur only in the specially grave cases—that is, in fatal cases. It is more reasonable, however, to think that they occur in all toxic-infective forms of a certain intensity, even in those in which the patient has recovered, and in which the lesions, being relatively superficial and mild, may possibly heal easily and permanently.

24. Michaelis and Blum: Deutsche med. Wchnschr. **24**:550, 1898.
25. Bernard and Salomon: Compt. rend. Soc. de biol. **57**:359, 1904.
26. de Vecchi, B.: Riforma med. **21**:96, 1921.
27. Mencarelli, L.: Cuore e circolaz. **14**:300, 1920.

It is this healing process that is of a special interest, since one cannot imagine a real restitutio ad integrum in such delicate tissues as those of the infant's valves, altered and damaged by the destructive and reactive processes, which usually involve the deeper layers of the segments. Possibly superficial valvular lesions, especially those confined to the endothelial and subendothelial layers, might heal quickly and completely, but deeper changes of focal necrosis and lymphocytic and histiocytic infiltration (sometimes very intense) could not disappear without leaving some trace. This is a theory that is readily applied, and Holsti [5] adopted it fully in explaining the pathogenesis of valvular inflammatory changes running a subacute and chronic course.

It is easy to understand, in view of the character of the changes, especially in view of the constant and marked histiocytic reaction, that when the general process has come to an end, healing can take place only by proliferation and substitution of connective tissue, with consequent deformity of the margins of the valves to an extent dependent on the severity of the primary lesions. When the inflammatory process has involved the deeper parts, marked changes must result.

CONCLUSIONS

From these researches one may draw the general conclusion that inflammatory processes of marked intensity often take place in young children affected by various toxic infective diseases, but that the subsequent process of thrombosis often fails to appear; thus the macroscopic diagnosis of such lesions at autopsy is almost always impossible. These are lesions of particular importance both for the inferences that can be drawn from them about the pathogenesis of the endocarditic process in general, and on account of the sequelae to which their further development gives rise, in the establishment of valvular defects the mode of origin of which has hitherto been obscure.

FAT NECROSIS IN BILE PERITONITIS

EXPERIMENTAL STUDY *

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During a recent study [1] of the reaction of the peritoneum to sterile bile, fat necrosis was frequently observed in the fat of the mesentery near the pancreas. The term fat necrosis has come to indicate a special form of necrosis of fat tissue which is characterized by a focal circumscribed lesion and by the hydrolysis of the fat in the necrotic area into fatty acids and glycerol, the latter disappearing and the former combining with bases to form soaps. In practically all cases, fat necrosis is produced by the action of pancreatic juice on the adipose tissue. A precise summary of the knowledge of this subject was made by Wells.[2]

Langerhans,[3] in 1890, observed that the fat in the cells is split into its components, and that the fatty acids combine (at least in part) with calcium to form insoluble soaps. Dettmer,[4] in 1895, was able to produce fat necrosis with fresh pancreatic juice, but was not able to do so with a commercial preparation of trypsin. He then concluded that the lipase of the pancreatic juice was the active agent. Flexner,[5] in 1897, supported this contention by demonstrating the presence of the lipase in the foci of fat necrosis. Opie [6] confirmed this observation and was able to demonstrate [7] the presence of a fat-splitting enzyme in the urine of a patient with fat necrosis.

Wells,[8] in 1903, produced typical fat necrosis by injecting extracts of fresh pancreas into animals either of the same species as that from

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* From the Department of Surgery of the University of Chicago.

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1. Rewbridge, A. G.: The Etiological Rôle of Gas Forming Bacilli in Bile Peritonitis, Surg. Gynec. Obst. **52**:205, 1931.

2. Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 431.

3. Langerhans, R.: Ueber multiple Fettgewebsnekrose, Virchows Arch. f. path. Anat. **122**:252, 1890.

4. Dettmer: Dissertation, Göttingen, 1895.

5. Flexner, S.: On the Occurrence of Fat Splitting Ferments in Peritoneal Fat Necrosis and the Histology of These Lesions, J. Exper. Med. **2**:413, 1897.

6. Opie, E. I.: Experimental Disseminated Fat Necrosis, Johns Hopkins Hosp. Rep. **9**:859, 1900.

7. Opie, E. I.: A Case of Hemorrhagic Pancreatitis: The Occurrance of a Fat Splitting Ferment in the Urine, Bull. Johns Hopkins Hosp. **13**:117, 1902.

8. Wells, H. G.: Experimental Fat Necrosis, J. M. Research **9**:70, 1903.

which the pancreas was obtained or of another species. The power of these agents to produce fat necrosis was reduced by heating to 60 C. for five minutes and destroyed at 71 C., indicating that the active agent was an enzyme.

Fat necrosis may be produced by any means that will cause the escape of pancreatic juice from its natural channels. It has followed trauma, acute infections of the gland and the blocking of the ampulla of Vater by gallstones, which permits bile to back up into the pancreatic ducts, where it results in an acute inflammation of the pancreas (Opie [9]).

Flexner,[10] in 1906, showed that it was the bile salts that caused the inflammation when bile passed up the pancreatic ducts, and that this effect was decreased or prevented by large amounts of colloids. As the result of injury by the bile salts, or any other agent that produces death of cells, the injured cells are digested by the pancreatic juice, which then escapes into the surrounding tissue. Wells' experiments showed that the lesions of fat necrosis may be produced in from three to five hours, large enough to be visible to the naked eye; their form and size depend solely on the area of fatty tissue exposed to the action of pancreatic juice.

Schweizer,[11] in 1924, reported a case of bile peritonitis which resulted from a spontaneous perforation of the gallbladder and in which fat necrosis was observed. At necropsy gallstones were present in the gallbladder and cystic duct, but not in the common bile duct or at the papilla of Vater. Both gross and microscopic examination of the pancreas failed to disclose any necrosis or evidence of an inflammatory process. He concluded from this that fat necrosis may occur in the abdomen quite independent of pancreatic disease or of the action of pancreatic juice. The following experiments reproduce the same pathologic changes in dogs that Schweizer observed in man.

METHODS

The animals, in the experiments to be described, were electrocuted eighteen hours after their operations. Necropsies were then performed, and the tissues were immediately placed in Zenker's fixative, later embedded in paraffin and stained with hematoxylin and eosin.

EXPERIMENT 1

In a series of twenty dogs, after double ligation of the common bile duct and the making of a stroma in the gallbladder, bile was allowed to drain continuously into the peritoneal cavity. Eighteen hours after

9. Opie, E. I.: The Etiology of Acute Hemorrhagic Pancreatitis, Bull. Johns Hopkins Hosp. **12**:182, 1901.

10. Flexner, S.: The Constituent of Bile Causing Pancreatitis and the Effect of Colloids on Its Action, J. Exper. Med. **8**:167, 1906.

11. Schweizer, R.: Fat Tissue Necrosis with a Perforated Gall Bladder, Schweiz. med. Wchnschr. **54**:265, 1924.

the operation the peritoneal cavity of each dog presented the picture of acute, severe general peritonitis and contained several hundred cubic centimeters of a serosanguineous exudate, in which were found polymorphonuclear leukocytes and *B. welchii.* The peritoneal surfaces were inflamed and were covered with a thin layer of fibrin. In the fat of

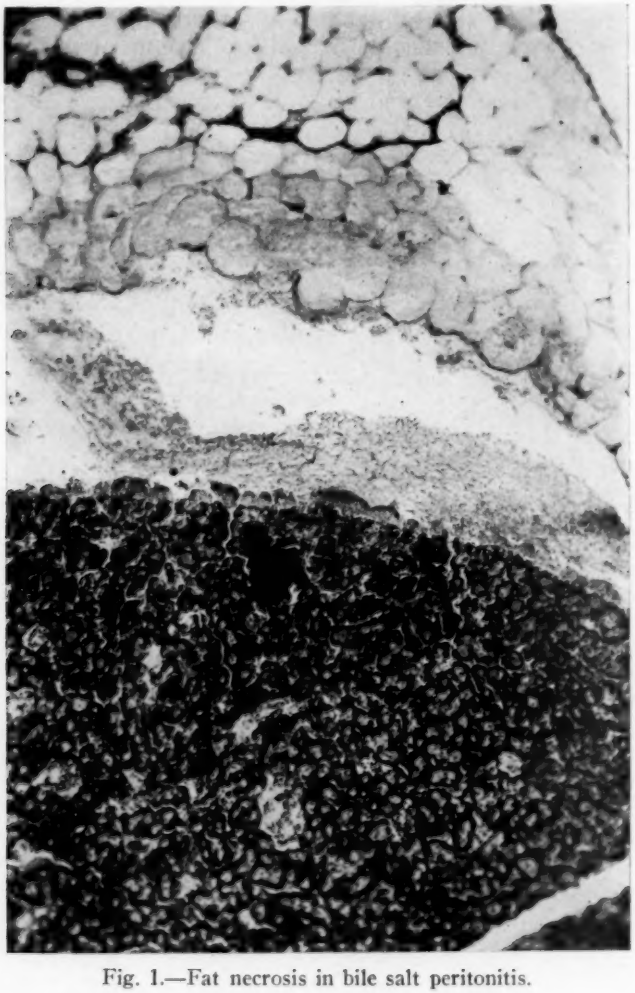

Fig. 1.—Fat necrosis in bile salt peritonitis.

the mesentery adjacent to the pancreas were areas of fat necrosis about 1 cm. in diameter. Microscopic examination of the pancreas failed to show any evidences of distention of the pancreatic ducts, necrosis of the glandular tissue or any inflammatory exudate in the gland or its stroma. Fat necrosis was observed in fifteen of the twenty dogs in this series.

EXPERIMENT 2

In a series of twenty dogs the effect of an intraperitoneal injection of a 10 per cent solution of bile salts was then studied. Eighteen hours after the administration of 2.5 cc. of a sterile solution of bile salts per kilogram of body weight there was observed a peritonitis similar

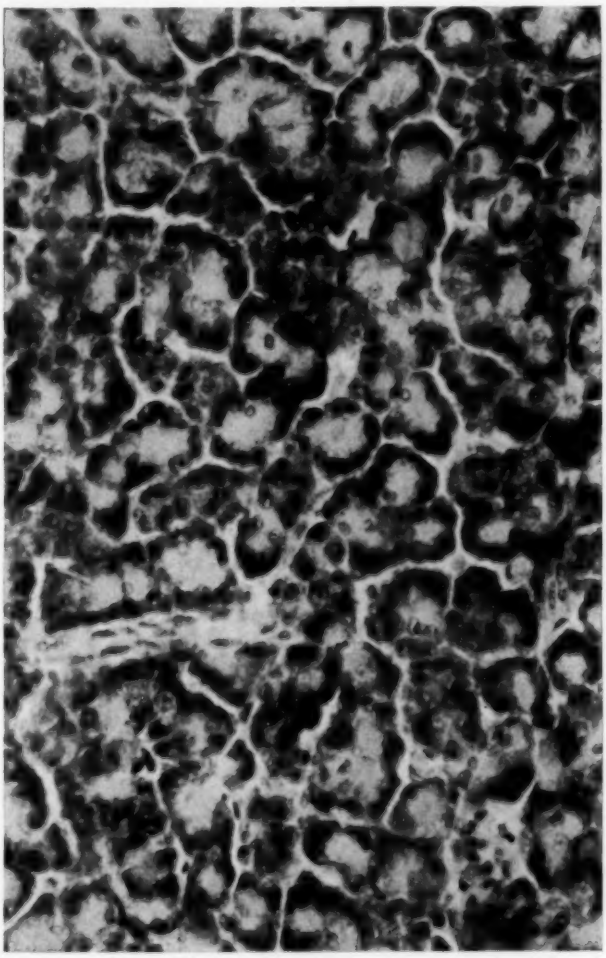

Fig. 2.—Normal pancreas near an area of fat necrosis.

to bile peritonitis, except that the fat necrosis was more extensive, being observed in the fat adjacent to the pancreas, in the mesentery, in the omentum and in the retroperitoneal fat. In this series of twenty dogs fat necrosis was observed in all instances.

The pancreas, when examined grossly, was normal. When examined microscopically no evidence of necrosis of the gland or of distention

of the pancreatic ducts was present. In the adipose tissue surrounding the pancreas were areas of fat necrosis and an occasional accumulation of polymorphonuclear leukocytes, which represented an inflammation secondary to the peritonitis. It is of interest to note, however, that this inflammatory process was confined to the fatty tissue, and that

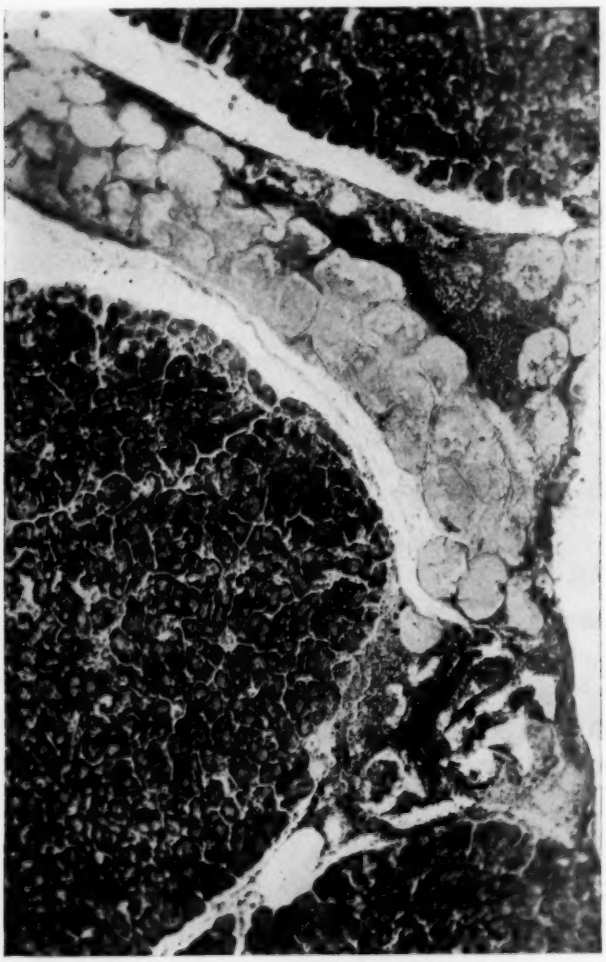

Fig. 3.—Fat necrosis in bile salt peritonitis.

normal pancreatic cells were adjacent to the foci of fat necrosis. The accompanying photomicrographs portray the condition of the pancreases of animals that had extensive areas of fat necrosis produced by the solution of bile salts.

Experiments 1 and 2, in which fat necrosis was observed when bile and a solution of bile salts were introduced into the peritoneal cavity,

tend to show that the bile salts were one of the important factors in its production. However, in the peritoneal exudates produced by bile and bile salts, *B. welchii* was found. In order to determine whether sepsis was involved in its production the following experiment was performed.

EXPERIMENT 3

In a series of six dogs peritonitis was produced by the intraperitoneal administration of 20 cc. of an eighteen hour broth culture of *B. welchii.* A peritonitis identical with bile peritonitis was produced, except that fat necrosis was not observed. This experiment tends to show that cultures of *B. welchii,* when introduced intraperitoneally, do not produce fat necrosis.

EXPERIMENT 4

The question arose as to whether the bile salts per se were able to produce fat necrosis, or whether the fat necrosis was produced by the local action of the bile salts on the pancreas liberating the pancreatic enzymes and allowing them to diffuse into the surrounding adipose tissue. In a series of six dogs the pancreas was completely excised before introducing intraperitoneally 2.5 cc of a 10 per cent solution of bile salts per kilogram of body weight. The amount of bile salts selected for this experiment had produced fat necrosis in every instance in experiment 2. Although a hemorrhagic peritonitis similar to that of bile peritonitis was produced, no fat necrosis was observed. As a control on this experiment the solution of bile salts was introduced intraperitoneally into three dogs which had had an operation of equal magnitude, such as a partial gastrectomy, and fat necrosis was observed in every instance. This experiment shows that the presence of the pancreas is essential for the production of fat necrosis by the intraperitoneal administration of bile.

COMMENT

In Schweizer's case of spontaneous perforation of the gallbladder, the fat necrosis was observed in the omentum. He concluded correctly that fat necrosis may occur independent of pancreatic disease. However, in view of the experiments described in the foregoing paragraphs, there is reason to doubt that it occurs independent of pancreatic juice, as stated by Schweizer. The evidence presented in this investigation shows that bile free in the peritoneal cavity is one of the factors involved in its production. Experiments with solution of bile salts show that the bile salts are the active agents in the bile. *B. welchii* was grown from the exudates produced by bile and bile salts; however, when peritonitis was produced by this organism, no fat necrosis was observed.

When only a few foci of fat necrosis were observed, these foci were always observed in the fatty tissue near the pancreas. When solution

of bile salts was introduced into the peritoneal cavities of depancreatized dogs, no fat necrosis was produced. The pancreases of the dogs with fat necrosis, when examined both grossly and microscopically, were normal. Previous investigations tend to show that fat necrosis is produced by extravasations of pancreatic juice permitting the enzymes to diffuse into the surrounding adipose tissue. Although there is no evidence to show that the bile salts per se are capable of producing fat necrosis, there is evidence to show that the presence of solution of bile salts free in the peritoneal cavity and the pancreas are essential for its production. Since there was no gross or microscopic evidence of necrosis of the pancreas in these experiments, it is necessary to postulate that the pancreatic enzymes were liberated by changes in permeability produced by the local action of the bile salts on the pancreas.

CONCLUSIONS

Fat necrosis may be produced experimentally by allowing a sufficient quantity of bile to drain into the peritoneal cavity.

The bile salts are the active agents in the bile.

The presence of the pancreas is essential for the production of fat necrosis by the intraperitoneal administration of bile.

The liberation of the pancreatic enzymes is produced presumably by changes in permeability caused by the local action of the bile salts on the pancreas.

MYOMA OF THE ESOPHAGUS WITH ASSOCIATED DIVERTICULA

REPORT OF TWO INSTANCES *

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Leiomyomas occur chiefly in preexisting smooth muscle tissue. In the intestine leiomyomas are not uncommon, but they are rarely seen in the stomach and esophagus.

The association of diverticula with subperitoneal myoma of the intestine was noted by Steiner,[1] but so far as I have been able to determine, such an occurrence has not been described in any of the few instances of esophageal myoma recorded in the literature.

It is true that these tumors are small, relatively rare and often asymptomatic, but the association with diverticula may add to their significance. Benign tumors of the esophagus may assume considerable importance, as in the instance reported by Fahr.[2] In this case the patient died as a result of obstruction to the esophagus by a long, sausage-shaped, pedunculated fibroma which was attached at the origin of the esophagus and extended to the cardia. Three examples of myomas of the esophagus are referred to by Ewing:[3] Pichler's case of multiple leiomyomas with striated muscle cells and ganglion cells, found in the muscular coat; Illig's case of a large myoma of the esophagus in the muscularis; and Eising's case of polypus myoma made up of atypical smooth muscle cells resembling sarcoma, which was also the seat of an early carcinoma. Another example of benign polypoid fibroma was reported by Dyke,[4] in which the patient died as the result of a malignant stricture of the esophagus. At autopsy a soft fibroma, covered with esophageal mucosa, was found attached at the level of the cricoid cartilage. Anitschkow [5] stated that of all fibromas and fibromyomas of the gastro-intestinal tract, the myomas, occurring in the muscular coat of the esophagus near the cardia, are the rarest. These, however, are not clinically significant. He referred to Pichler's case cited here and Ritter's case with malignancy. Anitschkow reported two instances of

* Submitted for publication, Jan. 14, 1931.

* From the Department of Pathology, University of Oregon Medical School.

1. Steiner: Beitr. z. klin. Chir. **22**:1, 1898.
2. Fahr, T.: Klin. Wchnschr. **2**:2347, 1923.
3. Ewing, James: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1927, p. 233.
4. Dyke, S. C.: J. Path. & Bact. **30**:309, 1927.
5. Anitschkow, N. N.: Virchows Arch. f. path. Anat. **205**:443, 1911.

small myomas of the esophagus just above the cardia. He believed that the most probable explanation of their occurrence is on an embryologic basis.

No reference is made to coexistence of diverticula with benign tumors of the esophagus. Jackson and Shallow [6] reported a case of diverticulum associated with a malignant condition of the esophagus.

In view of the fact that no instances of diverticula with related myomas have been recorded in the literature, it seems justifiable to report two cases.

REPORT OF CASES

CASE 1.—A woman, aged 32, died at the Multnomah County Hospital of latent corrosive mercuric chloride poisoning. She took poison with suicidal intent and was brought to the hospital in a moribund condition. The mercurial nephrosis had begun to subside when the patient died of diphtheroid and hemorrhagic ileocolitis. The small diverticulum and myofibroma in the lower third of the esophagus were discovered in the course of the routine postmortem examination. There was nothing in the clinical history to indicate that these had ever caused symptoms.

The tumor was 2.5 cm. above the cardia in the left lateral and posterior wall of the esophagus (fig. 1). It was kidney-shaped, with the long axis parallel to the vertical diameter of the esophagus. The concave surface was directed internally toward the lumen of the esophagus and impinged on the orifice of the diverticulum, which it narrowed. The tumor measured 3.5 cm. in length, 1.5 cm. in width and 1.3 cm. in thickness. The diverticulum extended cephalad and was partially surrounded by the tumor, which was arranged over the superior half. This association resulted in a comma-shaped ostium of the diverticulum with the narrow end directed downward. The opening here measured 0.5 cm. by 1.5 cm. The diverticulum was 1 cm. deep and 1.5 cm. wide, and extended 2 cm. upward. The tumor was externally smooth and circumscribed. The cut surface was firm, shiny and whitish.

To study the location and structure of the tumor and diverticulum better the esophageal wall was cut into blocks, each about 2 mm. thick. The blocks, indicated in figure 2 by the letters *A, B, C, D, E* and *F,* were embedded in paraffin and sectioned serially. Every tenth section was mounted, stained with van Gieson's connective tissue stain and counterstained with hematoxylin.

Block *A* included the most inferior portion of the tip of the comma-like orifice of the diverticulum. Grossly here the mucosa was eroded at the midpoint, then thickened in the depression where it was folded back near the inferior pole of the tumor. The muscular coats were of relatively uniform thickness. The end of the block near the tumor was thick, owing to the folding of the mucosa and a small accumulation of fat external to the muscular coats.

Microscopic examination of the serial sections revealed definite changes, particularly in the mucosa. The usual stratified squamous epithelium was seen at the narrow end of the section, but extended only about one fourth of the way across the surface to the point of infolding. For a short distance the mucosa was completely absent. Soon, however, there were seen glandlike structures in the scarred, exposed muscularis mucosae. The mucosa here was replaced by an amorphous fibrous and fibrinous substance staining dull blue, and containing many groups of alveoli and crypts. The latter were lined with disintegrating tall columnar cells

6. Jackson, C., and Shallow, T. A.: Ann. Surg. **83**:1, 1926.

Fig. 1 (case 1).—Esophagus opened along the midline posteriorly: *A* is the orifice of the diverticulum; *B*, the tumor in the lateral and posterior wall. The dotted line *C* indicates the extension of the myoma about the diverticulum.

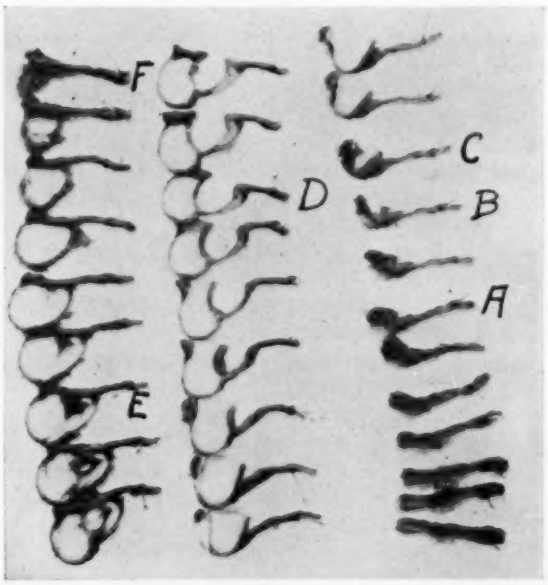

Fig. 2 (case 1).—Serial sections illustrating the relationship of the tumor to the diverticulum. The blocks marked *A*, *B*, *C*, *D*, *E* and *F* were selected for serial microscopic sections.

with small, circular, deeply basic-staining nuclei. The cytoplasm was pale and clear, and occasionally formed goblet cells. The alveoli and crypts increased in number as one approached the infolding at the lower end of the orifice to the diverticulum. Here the entire mucosa was formed by these structures with but little stroma. There was an irregular infiltration of small lymphocytes and a few plasma cells about the alveoli. Such areas closely resembled the structure of the mucous glands of the esophagus. Beyond the infolding the mucosa was similar to that described in the depths of the fold, but the stroma here was abundant and a dull light blue. In the muscularis mucosae at this point there was a localized increase in the smooth muscle. The cells were crowded together and tended to form whorls. The stroma here was relatively abundant. The muscularis elsewhere was unchanged. The muscular coats were of the usual thickness and free from any cellular infiltration.

Section *B* was taken from the wall of the esophagus immediately below the inferior pole of the tumor and prepared and stained as described. The infolding was deeper than in section *A*, and the erosion of the mucosa less pronounced. Microscopically the sections were similar to those of *A*. The glandular structure of the mucosa at the infolding was more pronounced. The mucosa in these sections simulated that of the small bowel. The stratified epithelium at the opposite end of the section covered more of the surface, was thinner, and in one section approached the glandular structures. Here the stratified epithelium was very thin and fragmented, and appeared to extend out over the glandular area. It was separated from the latter by a thin layer of fibrous connective tissue. It was evident that the glandular structure represented an overgrowth of the mucous glands in the muscularis mucosae, the entire mucosa being eroded. There was some distortion of the muscularis beyond the infolding similar to that seen in section *A*. However, the small tumor present here was lost in sectioning. The muscular coat was unchanged.

Gross section *C* was taken through the inferior pole of the tumor. Macroscopically the mucosa was more complete than in the other two blocks. The infolding was deeper, and the modified mucosa of the depression was thicker. The muscular walls appeared thicker about the tumor. The tumor here was located between the muscularis mucosae and the inner circular muscular coat. Microscopically the stratified epithelium was similar to that in section *B*. It covered more of the muscularis and in many sections extended to within a few millimeters of the infolding. The modified mucosa, made up of mucous glands resting on the muscularis, was more abundant and the fold deeper than previously described. Many lymph follicles were seen in the muscularis. The infiltration of the modified mucosa by small round cells was here as in other portions. The tumor at the wide end of the section was situated between the muscularis mucosae and the inner muscular coat. In two of the serial sections the smooth muscle cells of the tumor were seen to be continuous with the inner surface of the inner circular muscle bundles. The tumor was very dense, being made up of closely packed smooth muscle cells and fibrous connective tissue. The smooth muscle cells predominated. These cells were arranged in parallel bundles with an occasional whorl. At this end of the tumor there was an infolding from the inferior surface, leaving a small opening and an incomplete septum partially dividing the main mass. In the small opening, down near the muscular coat, there were many sinuses filled with blood. These formed a small area of hemangiectasis within the base of the tumor. The tumor was loosely encapsulated and was itself relatively avascular. The muscular coat at this point was many times the thickness seen in sections *B* and *A*. There were no other evident microscopic changes.

Block *D* was taken through the upper end of the tumor at the widest portion of the orifice of the diverticulum. Here the tumor was situated in each lateral wall of the diverticulum. The main mass was found at the extreme edge of the section with a small, thin comma-shaped portion in the opposite wall. The mucosa appeared to be thin as it dipped into the diverticulum. The muscular wall was not grossly evident about the walls of the diverticulum.

Microscopically the stratified squamous epithelium was thinned and eroded, but covered the flat surfaces and dipped down into the diverticulum covering almost the entire wall. The floor of the diverticulum, however, was devoid of an epithelial lining. The muscularis was exposed, fibrosed and continuous throughout, but thinned as it dipped down into the walls of the diverticulum. The muscular coat extended to the edge of the small comma-shaped tumor, then became very thin as it extended around the tumor and diverticulum. This small tumor was continuous with the inner layer of smooth muscle and was similar in appearance to previous sections of it. The large tumor mass in the opposite wall was of like structure, having an avascular central portion and a somewhat vascular periphery.

Block *E* was taken through the larger portion of the tumor and the most superior tip of the diverticulum. Here the diverticulum extended to the mucosa, muscularis and muscular coat, as it extended cephalad from the ostium. It was surrounded on three sides by tumor. The mucosa and walls of the esophagus appeared grossly unchanged. The tumor and diverticulum were between the deep layer of the muscular coat and the periesophageal connective tissue. Microscopically the mucosa, muscularis mucosae and muscular coats were unchanged. The serial section of the small, pouchlike tip of the diverticulum revealed it to be lined with stratified squamous epithelium which was slightly thinner than that seen on the surface of the esophagus, but which was otherwise unchanged. The muscularis was seen deep to the ring of stratified squamous epithelium. The tumor was essentially as described previously.

Gross section *F*, the last section of the series, was taken at the most superior tip of the tumor. Grossly no changes were observed in the wall of the esophagus, except for a slight thickening of the mucosa overlying the tips of the tumor. Microscopic sections revealed the presence of several mucous glands beneath the mucosa. These had slender ducts, which passed along obliquely through the muscularis and mucosa. Just proximal to the orifice was an ampulla, which narrowed down to form an orifice of small caliber. Near the thick mucosa over the tumor the ampulla of one duct was dilated, being some five times the size of others. The duct was also dilated, and the gland from which it came was slightly enlarged. Here also the overlying mucosa was thrown into small folds and was scarred. The glands seen in the other portions were located in the loose connective tissue between the muscularis and the muscular coat. The structure of these glands was identical with the modified mucosa occurring in the fold at the orifice of the diverticulum. There were no remarkable changes in the muscular coat.

Case 2.—A woman, 59 years of age, was found dead at her residence. The coroner's autopsy revealed marked coronary arteriosclerosis, thrombosis of the anterior interventricular branch of the left coronary artery and chronic rheumatic mitral endocarditis with stenosis and insufficiency. During the course of the necropsy a small myoma was discovered in the posterior wall of the lower third of the esophagus. Just above the tumor in the anterior wall there was a shallow diverticulum.

The tumor was located 1.2 cm. above the cardia and was 1.2 cm. in diameter and 1.3 cm. long. It was bean-shaped, with a twisted tapering projection from

right to left in the posterior wall of the esophagus. The diverticulum was 3.5 cm. above the cardia, was oval shaped, and measured 1.5 cm. long, 1 cm. wide, 0.5 cm. deep. The long axis was parallel to the vertical diameter of the esophagus. The esophagus was 4.5 cm. in circumference at the cardia, 3.5 cm. at the tumor and 4 cm. just above the tumor. Gross serial sections were made of this portion of the esophagus and were prepared as in the previous series. The sections selected for

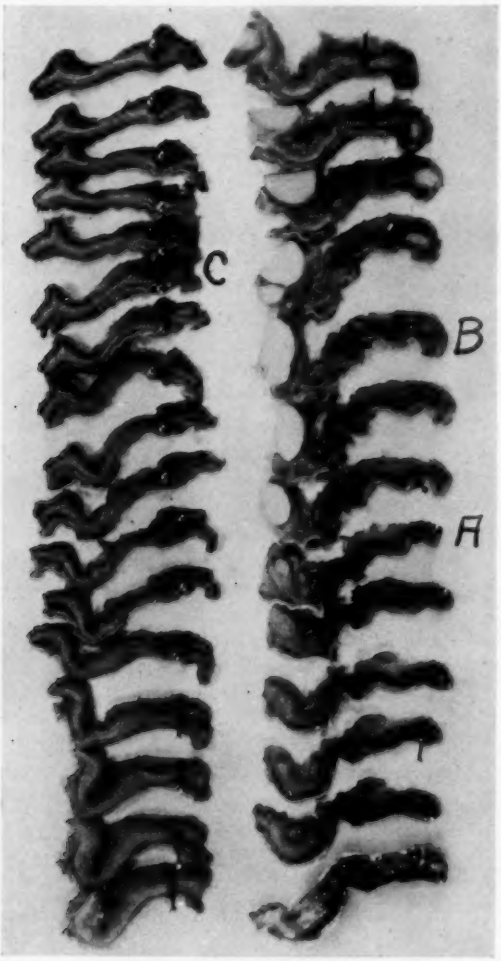

Fig. 3 (case 2).—Cross-sections of the esophagus, tumor and shallow diverticulum. The blocks between *B* and *C* show the relationship of the tumor and diverticulum. Note that the association is not as close as that in case 1 (fig. 2).

serial microscopic sections are marked *A*, *B* and *C* in figure 3. Block *A* was from the most inferior pole of the tumor. The mucosa here was wrinkled and intact. The tumor was located in the muscular coat. Microscopic examination of serial sections revealed the mucosa about the tumor to be modified. Here the stratified epithelium was thinned and eroded. For a short distance the stratified squamous

epithelium was absent, and a modified mucosa similar to that described in the fold in case 1 was seen. Likewise there was a large dilated duct, lined with tall columnar epithelium. The muscularis was intact throughout. Between it and the muscular coat were many mucous glands and ducts, as well as an occasional lymph follicle. The tumor at one end of the section was similar in structure to that seen in case 1. It was made up of smooth muscle, a scant stroma and blood supply. At one point the fibers of the tumor were continuous with those of the internal muscular coat. No further changes were noted in the esophageal wall.

Block *B* was taken from the tip of the small twisted end of the tumor. Grossly the mucosa was intact, and the small tumor was indistinctly seen in the muscular coat. Microscopically, the stratified squamous epithelium was intact, except over the tumor, where glandular structures occurred. The mucous glands of the muscularis mucosae were numerous and were slightly infiltrated with small round cells. The mucosa was thrown into folds and at one point formed a small pouch, which was very near the tip of the tumor. Beneath it the tissue was slightly infiltrated with small round cells and was fibrosed. The tumor in this location was small and completely surrounded by the muscular coat. The latter contained in one section another mass similar to the tumor, but much smaller.

Gross section *C* was taken through the shallow diverticulum above the tumor. Macroscopically the mucosa appeared folded, but otherwise negative. The muscular walls were unchanged. Microscopically the stratified squamous epithelium was intact, but was thinned in the region of the diverticulum. The muscularis mucosae was intact and unchanged. The muscular coats revealed no microscopic changes.

COMMENT

In both cases the leiomyomas occurred in the inner layer of the smooth muscle and were directly connected with it. The small tumors seen in the second instance were embedded in the smooth muscle, were circumscribed and exhibited pseudo-encapsulation. These tumors were made of orderly whorls of smooth muscle cells very different from the normal wavy smooth muscle about them. Such characteristics, with the finding of other small tumors similar in structure and location to the larger ones, pointed to their congenital origin. Congenital anomalies are known to be multiple rather than singular.

The areas of muscularis covered with mucous glands simulating gastric mucosa described in the sections from both cases probably represented another anomaly. Ewing,[7] in discussing the etiology of carcinoma of the esophagus, referred to islands of mucous glands in the esophageal mucosa of persons showing this condition. Hewlett [8] described these as the superficial glands of the esophagus and considered them to be distinct from the mucous glands beneath the muscularis. He stated that these occur as islands of branched tubular glands lined with columnar epithelium, replacing the stratified squamous epithelium. Microscopically they simulate gastric mucosa, but as the esophageal

7. Ewing (footnote 3, page 904).

8. Hewlett, A. W.: J. Exper. Med. **5**:319, 1900-1901.

mucosa is considered by Maximow to develop in situ, they could not be congenitally misplaced gastric mucosa rests. The absence of parietal cells is another point against the gastric origin. Hewlett further stated that such islands of glands occur most commonly at the level of the cricoid cartilage, but the cardiac end of the esophagus may contain them.

The association of leiomyomas diverticula and islands of superficial glands of the esophagus undoubtedly points to a common congenital origin. Diverticula are known [6] to develop at certain ill-supported areas of the esophagus. Others are reported to be of congenital origin. Jackson and Shallow [6] reported such a case and stated that Maylord believed that all congenital diverticula of the esophagus have their origin in persistent second branchial clefts. Steiner [1] found that the associated changes in the lumen of the small intestine depended on the location of the tumor in the wall of the bowel. The submucous type cause stenosis or intussusception, and the subperitoneal produce diverticula. These diverticula associated with the small myomas of the esophagus may be the result of mechanical factors coupled with the congenital defects, evidenced by the tumors and the superficial glands. The enlargement of the tumor and the downward pressure of swallowing may cause a rotation of the tumor with eccentric enlargement and a drawing in of the esophageal mucosa to form the diverticulum. Other factors may be the thinning of the wall about the tumor and the possibility of a congenital defect of the wall as a fourth anomaly.

SUMMARY

Two instances of myoma of the lower third of the esophagus with associated diverticula are reported, with their gross and microscopic characteristics, and illustrations are given.

HEALING OF THE GASTRIC MUCOUS MEMBRANE IN RABBITS AFTER ITS SURGICAL REMOVAL*

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The present investigation of the healing of experimental lesions of the gastric mucous membrane in rabbits was begun to determine whether there is a tendency in these animals toward the development of chronic gastric ulcer and to consider the mechanical effects of the continuous presence of food in a constantly distended stomach.

Ferguson [1] removed an area of mucosa about 1 cm. in diameter from the middle of the anterior surface of the stomach in rabbits, and allowed them to live for from one to forty days after the operation. There were few signs of regeneration, and an ulcer was found in every rabbit of the experiment. He suggested that the rabbit's stomach is never empty, that the underlying suture in the muscular and serous coats acts as a foreign body, and that there is an intrinsic lack of regenerative ability in the gastric epithelium.

Continuing the work begun by Ferguson, Fauley and Ivy [2] removed an area from the posterior, and one from the anterior, wall of the stomach in rabbits, through an incision in the anterior wall. In thirty days the simple lesion of the posterior wall had healed, but there was a chronic ulcer on the anterior wall at the site of the silk suture. These rabbits were fed normal rough food. In spite of the silk suture, however, only three of seventeen rabbits which were fed soft food had ulcers thirty days after the removal of areas of mucosa from the anterior walls of their stomachs.

Nicolaysen [3] froze an area in the anterior gastric wall in rabbits with ethyl chloride for from three to eight minutes. This injured all layers, not merely the mucosa. Most of the animals that lived, some for six months, had typical chronic peptic ulcers.

METHOD OF EXPERIMENTS

In operating on the rabbits, aseptic technic and ether anesthesia were used. The animals were allowed a diet of alfalfa hay, oats and water, with no restriction of the quantity eaten. Two types of operation were performed: removal of an area

* Submitted for publication, Dec. 9, 1930.

1. Ferguson, A. N.: Am. J. Anat. **42**:403, 1928.

2. Fauley, G. B., and Ivy, A. C.: Proc. Soc. Exper. Biol. & Med. **27**:531, 1930.

3. Nicolaysen, Knud: Deutsche Ztschr. f. Chir. **167**:145, 1921.

of mucous membrane from the region of the fundus and removal of an area of mucous membrane from the region of the pylorus.

Removal of Area from the Fundus.—The abdomen was opened in the median line, from the xiphoid process to the umbilicus. Two mosquito forceps were attached to the anterior wall of the stomach, one near the greater curvature at about its middle, the other 3 cm. from the first, toward the lesser curvature. The stomach was lifted up into the incision with these forceps, and the remainder of the abdominal cavity was carefully protected by moist gauze packs. The serous and muscular coats were incised between the points at which the forceps were attached. By retracting the combined serous and muscular coats, the underlying mucous membrane was readily exposed over a large area by blunt dissection. Then, with scissors, a circular piece of mucous membrane was removed, and the opening into the stomach was immediately packed with gauze to stop hemorrhage and to prevent leakage of gastric contents. Usually a piece of gastric mucosa about 2.5 cm. in diameter was removed; however, the size could not be determined exactly because the mucosa always seemed to stretch as it was being exposed. The incision in the wall of the stomach was closed by one layer of continuous suture through the combined serous and muscular coats. In some of the experiments silk sutures were used; in others, chromic catgut (no. 00), and in still others, plain catgut (no. 00). The omentum was always laid over the suture line in the stomach. The incision in the abdominal wall was closed with three layers of continuous silk suture.

Removal of Area from the Pylorus.—The abdominal incision was the same as in the first procedure. Two mosquito forceps were attached to the anterior surface of the pyloric portion of the stomach, one at the upper edge of the thickened band of pyloric muscle; the other, 2 cm. from the first, toward the fundus. With a small scalpel, an incision 2 cm. long was made between the two forceps through all layers of the gastric wall, and the mucous membrane on the lesser curvature at the upper edge of the pylorus was grasped in the forceps. By lifting gently on the forceps, the mucous membrane was separated from the underlying muscle, and a round area was snipped off with scissors. Areas from 0.5 to 1.2 cm. in diameter were removed. The incision in the gastric wall was sutured with plain catgut (no. 00) to make a transverse closure; the mucosa was closed with a continuous suture, and the muscle and serosa were inverted with a mattress suture. The incision in the abdominal wall was closed as in the first procedure.

RESULTS

Twenty animals used in the first series of experiments lived longer than 20 days, and were dispatched at intervals of from 21 to 121 days after operation. One animal died of pneumonia and empyema 100 days after operation, but as the mucosa was completely healed, the experiment is included in the consideration of results. Practically all of the rabbits that were allowed to live for a long time gained weight and seemed healthy.

The gastric mucous membrane of nine of the twenty-one rabbits was found to be completely healed. Twelve rabbits had chronic gastric ulcer at the site of operation (table 1). All of these lesions, however, showed definite evidences of healing. The ulcers were much smaller than the original pieces of mucous membrane removed. None was

greater than 1 cm. in diameter; some were relatively deep and had perforated the muscular coat. The omentum was usually adherent to the serosa at the site of the lesion. The margins were slightly elevated, and overhung the base of the ulcer. On microscopic examination of histologic sections, regenerating epithelium was seen at the edges of the ulcers, as reported by Caylor [4] in his description of the healing ulcer of human beings. The general picture of these gastric ulcers in rabbits is similar to that described by Mann [5] for healing gastrojejunal ulcers in dogs.

The shortest time in which complete healing occurred was forty-nine days. For purposes of comparison, the animals that were killed

TABLE 1.—*Results After Excision of an Area of Mucous Membrane from the Fundus of the Stomach*

Rabbit	Time Allowed to Live After Operation, Days	Dimensions of Ulcer, Cm.*	Type of Suture in Wall of Stomach
1	21	0.8 by 0.8 by 1.0	Silk
2	27	0.6 by 0.6 by 0.5	Silk
3	28	0.3 by 0.3 by 0.2	Plain catgut
4	28	0.8 by 0.5 by 0.3	Plain catgut
5	33	0.3 by 0.3	Silk
6	49	None	Silk
7	50	None	Plain catgut
8	57	0.4 by 0.4 by 0.4	Silk
9	57	0.4 by 0.3 by 0.2	Chromic catgut
10	64	None	Silk
11	64	None	Plain catgut
12	71	None	Chromic catgut
13	72	None	Silk
14	74	0.8 by 0.8 by 1.0	Silk
15	85	0.2 by 0.1 by 0.1	Plain catgut
16	91	1.0 by 0.8	Silk
17	99	None	Chromic catgut
18	100	None	Silk
19	101	0.3 by 0.3	Silk
20	106	0.2 by 0.1 by 0.1	Chromic catgut
21	121	None	Silk

* The area of gastric mucosa removed was approximately 2.5 cm. in diameter.

after forty-nine days were considered separately in groups according to the type of suture material used in closing the wall of the stomach. In each group, healed lesions and ulcers were about equal in number to those in the other groups, indicating that the type of suture used in the wall of the stomach apparently had no relation to the failure of the mucosa to heal (table 2). Also, although in some instances a piece of silk suture was hanging in the lumen of the stomach from the base of the ulcer, in others, pieces of silk suture extended through healed mucous membrane. It is certain that some of the ulcers were not caused by the presence of the suture as a persistent mechanical irritant,

4. Caylor, H. D.: Ann. Surg. **86**:905, 1927.

5. Mann, F. C.: S. Clin. North America **5**:753, 1925.

and that some of the areas healed in spite of the retained suture material.

Of the rabbits used in the second experiment, nine were killed at intervals of from 25 to 114 days after operation. In two rabbits, killed, 25 and 43 days after operation, respectively, ulcers were found at the sites of the removal of mucosa. In all the other animals of the series, the mucous membrane of the lesser curvature of the stomach was healed; in four of them, however, a chronic ulcer was present at the site of the gastrotomy (table 3). In all the operations of this type, the incision in the stomach was closed with sutures of plain catgut (no. 00).

TABLE 2.—*Relation of Type of Suture in Wall of Stomach to Healing*

Days After Operation	Silk		Chromic Catgut		Plain Catgut		Total	
	Healed	Ulcer Present	Healed	Ulcer Present	Healed	Ulcer Present	Healed	Ulcer Present
0 to 48	0	3	0	0	0	2	0	5
49 to 121	5	4	2	2	2	1	9	7
Total	5	7	2	2	2	3	9	12

TABLE 3.—*Results After Excision of an Area of Mucous Membrane from the Pyloric Region of the Stomach*

Rabbit	Time Allowed to Live After Operation, Days	Dimensions of Area of Mucosa Removed, Cm. (Approximately)	Dimensions of Ulcer	
			At Site of Removal of Area of Mucosa, Cm.	At Site of Gastrotomy, Cm.
22	25	0.5 by 0.5	1.0 by 0.2 by 0.1	None
23	43	1.2 by 0.7	1.0 by 0.8 by 0.2	None
24	50	1.0 by 0.8	None	1.2 by 0.7 by 0.2
25	84	0.5 by 0.5	None	1.0 by 0.7 by 0.1
26	88	1.0 by 0.5	None	None
27	105	0.8 by 0.8	None	1.5 by 0.5 by 0.2
28	105	1.0 by 1.0	None	None
29	114	1.0 by 0.8	None	0.1 by 0.1 by 0.1
30	114	0.8 by 0.8	None	None

Therefore, failure of the mucosa to heal completely was not due to the presence of nonabsorbable suture material in the underlying tissues. In one animal the lesion of the lesser curvature was entirely healed, and the mucosa had healed at the point of gastrotomy to form a tight sheath around several stiff hairs which were projecting into the stomach.

COMMENT

After removal of an area of mucosa from either the region of the fundus or that of the pylorus of the stomach of a rabbit, healing occurs much more slowly than in dogs or cats. Experiments involving the latter two animals have been reported by Morton,[6] Carnot,[7]

6. Morton, C. B.: Ann. Surg. **85**:207, 1927.
7. Carnot, Paul: Compt. rend. Soc. de biol. **94**:637, 1926.

Ferguson, Dragstedt,[8] Dragstedt and Vaughn[9] and Wilensky and Geist.[10] This was indicated by Ferguson, whose studies did not extend over so long a time after operation as the experiments now being reported.

As the areas of mucosa removed from the region of the pylorus were smaller than those removed from the region of the fundus, the rates of healing could not be exactly compared. Healing occurred more quickly, however, in the area in the lesser curvature of the stomach, in the region of the pylorus. Morton showed, in dogs, that this region is most susceptible to the development of chronic ulcer. Either this rule does not hold true in regard to rabbits, or some other factor was responsible for the development of chronic ulcers in the present experiments.

The presence of the suture line directly underneath the lesion of the gastric mucosa undoubtedly was significant in delaying healing. This was suggested in the series of operations on the fundus, but was more forcibly indicated in the operations in which an area was removed from the lesser curvature, near the pylorus. Chronic ulcers developed in almost half of the latter cases, under the scar of the incision, even though mucous membrane was not removed from that site. The presence of hair and other roughage in the gastric content and the presence of nonabsorbable suture material in the wall of the stomach did not seem to be significant factors in delaying healing. Probably the increased connective tissue at the site of the incision, resulting in impaired circulation, was the chief cause of the development of ulcers.

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8. Dragstedt, L. R.: Contributions to the Physiology of the Stomach: XXXVIII. Gastric Juice in Duodenal and Gastric Ulcers, J. A. M. A. **68**:330, 1917.

9. Dragstedt, L. R., and Vaughn, A. M.: Gastric Ulcer Studies, Arch. Surg. **8**:791, 1924.

10. Wilensky, A. O., and Geist, S. H.: Experimental Studies in the Production of Chronic Gastric Ulcer, J. A. M. A. **66**:1382, 1916.

Clinical and Occasional Notes

A NEW BRAIN SLICER*

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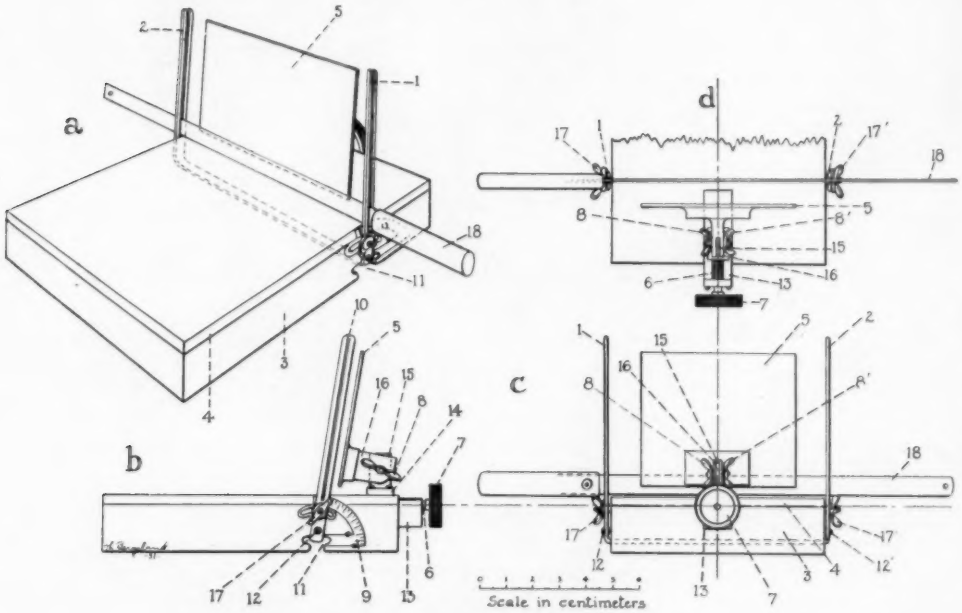
The device which is here described makes possible the accurate cutting of uniformly thick gross sections of the hardened human brain, varying in thickness from 5 mm. to several centimeters. By orienting the brain with reference to the plate, *5* (see figure, *a*), sections may be cut in the horizontal, vertical (sagittal) and transverse (coronal) planes. Owing to the fact that the longitudinal axis of the brain is a curve with



Working drawing of brain slicer, one-seventh actual size.

the concavity downward, it is desirable to be able to cut transverse sections at right angles to this curve. This may be achieved by angulating the knife with successive sections through the brain from the anterior to the posterior portion. The plate, *5* (see figure), is essential for the cutting of thin sections, because it forms a firm, flat surface against which the brain is pressed. In order that the plate may be used when the knife is angulated, it is evident that the plate also must be capable of rotation, so that its surface may be kept parallel with that of the knife.

* Submitted for publication, April 9, 1931.

* From the Institute of Pathology, Western Reserve University.

The apparatus which is shown in different views in the figure (*a*, *b*, *c*, *d*) consists, in essentials, of two slotted knife-guides, *1* and *2*, which are mounted on an oak base, *3*, covered with linoleum, D. A brass plate, *5*, can be advanced or withdrawn by means of the screw, *6*, which is turned by knurled head, *7*. Angulation of the brass plate, *5*, is brought about and maintained by means of two winged nuts, *8* and *8'*. The degree of angulation of the knife-guides is measured on the protractor, *9*, and fixed by the winged nuts, *17* and *17'*. The plate is made parallel to the knife by sighting through the slots, *10*, of the knife-guides. The slotted knife-guides are made from one piece of strap iron, 5 by 12 mm. in cross-section, which is bent in the form of a broad letter U. The horizontal part of this piece of metal is placed in a slot, *11*, cut out of the under surface of the oak base, *3*. The axis about which the knife-guides rotate is that of the screws, *12* and *12'*. The dimensions of the base are 27.5 by 20 by 5 cm. The covering of linoleum is 6 mm. thick. The knife-guides are 20 cm. long. The slot in the knife-guides is 2 mm. wide. The screw, *6*, permits a travel of 6.3 cm. This screw, which is 1 cm. in diameter, is housed in an oblong brass box, *13*, 8 by 3.5 cm., made of a brass plate 0.5 cm. thick. A brass block, *14*, which travels on a screw, *6*, carries a brass tongue, *15*, which is placed in a narrow cleft of *16*, and gripped tightly by nuts *8* and *8'*. The plate, *5*, is made of brass 15 by 12.7 by 0.2 cm. The fiber of the wood is filled with melted paraffin to prevent absorption of liquids. The knife, *18*, which is 34 by 2.5 by 0.1 cm., was made from a piece of hack-saw blade of Sheffield steel.

Another very simple device to aid in slicing the brain was described by Anderson.[1]

1. Anderson, J.: How to Stain the Nervous System, Edinburgh, E. & S. Livingstone, 1929.

Historical Review

CARDIOVASCULAR RENAL DISEASE

REPORT OF A CASE OF THREE THOUSAND YEARS AGO *

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Although a good deal of work has been done on the gross anatomy and histology of mummies, the nature of the material has not usually been such that the diagnosis of a disease affecting several organs has been possible. The case here reported seems to be an example of the condition commonly called cardiovascular renal disease, occurring in an elderly person 3,000 years ago, who also had marked pneumonokoniosis, with encapsulated caseous areas in one lung.

Through the courtesy of Mr. H. E. Winlock of the Metropolitan Museum of Art, New York, the organs of seven Egyptian mummies were received in this laboratory. They were excavated from a tomb used for women connected with the royal family during the Twenty-First Dynasty (about 1,000 B.C.), at Deir el Bahri in ancient Thebes.

The mummy in question was that of a person named Teye, whose age, according to Mr. Winlock, seemed to be about 50 years. The hair was white, the skin of the abdomen wrinkled, and the bones were much rarefied, but the manubrium was not joined to the sternum.

Eight organs were submitted for examination. Of the eight it was possible to identify beyond question both lungs, the intestines, the kidneys, the heart and the aorta.

The heart was the most satisfactory. It needed to be handled with great care. It was about the size of a hen's egg, not much thicker than an egg shell and much more fragile. The mitral valve was located and photographed; the chordae tendineae and papillary muscles were plainly shown (fig. 1). There was a small calcified mass on one leaflet indicating old endocarditis.

The coronary arteries showed well marked fibrous thickening, chiefly of the intima, with good-sized patches of calcification (fig. 2). The striae on the cardiac muscle fibers were photographed. There were areas of fibrous tissue in the cardiac muscle, like little scars. It

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* From the Department of Pathology, University of Buffalo School of Medicine.

is probable that the woman suffered from cardiac pain that would be called angina pectoris today.

The aorta showed moderate nodular arteriosclerosis. Sections of the aorta took specific stains for elastic tissue well.

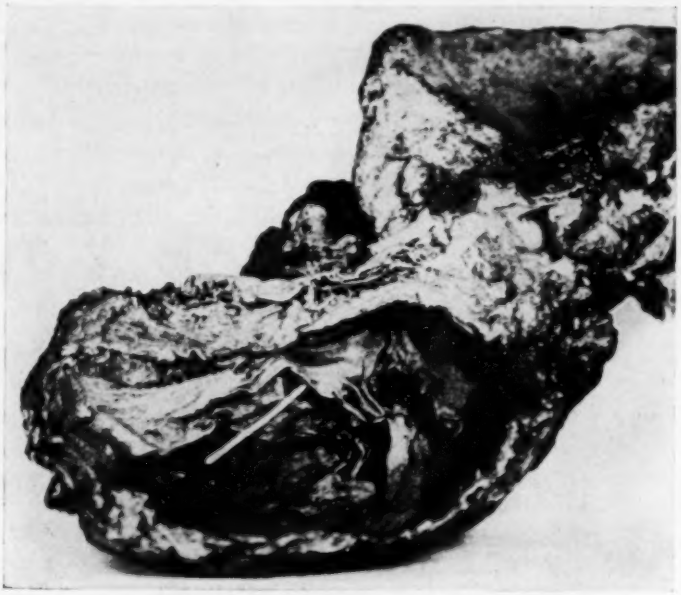

Fig. 1.—The heart, showing the mitral valve; natural size.

Fig. 2.—A coronary artery: a low power photomicrograph; × 16.

Both lungs presented marked pneumonokoniosis. Bits of cartilage from the bronchi were demonstrable. One lung had a few areas of caseation that were well encapsulated by fibrous tissue, but no tubercle bacilli were demonstrable.

The microscopic structure of the intestinal wall was recognizable only as far as its layers were concerned. The contents were miscellaneous vegetable fibers of no particular interest. No parasites were found.

Both kidneys were recognized by their histologic structure; many glomeruli were fibrous. There was a thick capsule and a large amount of interstitial fibrous tissue with arteriosclerosis of medium-sized arteries. No nuclear stain was secured in any of the sections.

In the package that should have contained liver were found two large pieces of tissue that appeared to have been varnished. Neither of them appeared like the livers from the other mummies. Each of these pieces contained a large mass of adipocere. In the examination of a large number of sections, no clue to the identity of the organ could be discovered. Only a quantity of adipose tissue could be recognized. The suspicion may be entertained that the ancient embalmers lost the liver belonging to the lady Teye, and that a mass of putrid tissue from some animal was substituted. It is also suggested that she may have been more than 50 years of age.

General Review

BACTERIAL METABOLISM, C

A SUMMARY *

ARTHUR ISAAC KENDALL

CHICAGO

Bacteria, the smallest and simplest in structure of known independent living things, are very close to that shadowy hiatus between the quick and the dead. In them the phenomena of life are reduced to the most primitive terms. They have no sex, no morphologically distinct nucleus,[1] and all the essential functions of growth, multiplication and purpose in the scheme of life are carried on by single cells, the average diameter of which is rather less than 1 micron (0.001 mm.), and of which a single one would weigh about 0.000,000,002 mg.[2]

In the study of the microscopic forms known as bacteria, we have what might fitly be called the focal point of the various branches of biological science. Though their investigation may require careful morphological researches, yet the unmistakable monotony of form, combined with a considerable variation of physiological activity, has compelled the bacteriologist to pay much attention to means by which such physiological variations may be more or less accurately registered in order that they may serve as a supplementary basis for classification. Again, with the unicellular organisms, the manifestations of cell activity become the most important phenomena for study. These manifestations bring together the fields of Physiology and Chemistry, and make Bacteriology in one sense a branch of Physiological Chemistry.[3]

Thus wrote Theobald Smith thirty-eight years ago. At that time, physiologic chemistry was a promising department of physiology, and bacteriology was making its spectacular appearance in the world of science as a foster child of pathology. Now, after four decades, the

* Submitted for publication, Jan. 21, 1931.

* From the Department of Research Bacteriology, Northwestern University Medical School.

* Studies in Bacterial Metabolism have appeared as follows: I-VII, J. Biol. Chem. **12**:13, 19, 215, 219, 465, 469, 1912; ibid. **13**:63, 1912; VIII, Med. Rec. **84**: 151, 1913; IX, Boston M. & S. J. **168**:825, 1913; X, J. Infect. Dis. **13**:425, 1913; XI, J. Biol. Chem. **15**:277, 1913; XIII, J. M. Research, n. s. **23**:465, 1913; XIII-XXX, J. Am. Chem. Soc. **35**:1201, 1913; XXXI-XXXVIII, ibid. **36**:1937, 1914; XXXIX-XCIX, J. Infect. Dis. **15-47**: (incl.), 1914-1930.

1. The bacterial cell is rich chemically in nuclear substance, however.

2. Kendall, A. I.: Civilization and the Microbe, Houghton Mifflin Company, 1923, p. 17.

3. Smith, Theobald: The Fermentation Tube, Wilder Quarter Century Book, 1893.

import of these prophetic asseverations has become more generally comprehended. The amazing developments in physiologic chemistry and in bacteriology have opened vistas which lead forward to the understanding and the conquest of disease, and to the harnessing of microbic activity to the projects of man. They reach backward to a primitive pattern of life itself. The chemical changes induced by various kinds of bacteria in their environment during growth and multiplication afford important information of the chemical pattern of life in general, and in particular as it relates to animals and to man.

GENERAL PHENOMENA OF BACTERIAL METABOLISM

The metabolism of any living organism consists of two distinct phases: a structural phase in which the organism reproduces and replaces its traditional and hereditary architecture, and an energy phase during which the organism characteristically performs certain actions consonant with its place on the ladder of life. In the bacteria these two phases, while somewhat overlapping, are readily distinguishable chemically, with respect both to the character of the products formed and to the nature of the processes involved. The structural phase precedes the energic phase in point of time, but this interval may be very brief indeed. For example, with the aid of the microscope, cholera vibrios, growing under optimal conditions of temperature and nutrition, may actually be seen to divide into two mature organisms every fifteen minutes. This rate of multiplication, if continued under favorable conditions, would mean ninety-six generations in twenty-four hours, with a theoretical daily progeny of nearly $8 \times 10,^{28}$ an immense number: thus a person may be exposed to cholera and die of it within eighteen hours. Fortunately, many natural barriers—exhaustion of food, accumulation of waste products, mutual antagonism—restrain microbic growth to compatible limits.

The phenomena of this microbic growth are important. Phylogenically, the cholera organism creates an exact replica of itself every fifteen minutes over a period of time. This involves among other substances, the fabrication of that exquisitely complex and specific hereditary protein architecture in which resides the élan and the individuality of the organism. Chemically, this proteinogenesis is a series of hydrogenic condensations in which simpler amino-acids and peptides are elaborated into complex specific proteins through the union of the smaller molecules, usually with elimination of hydrogen and oxygen in proportion to form water.[4] The actual amount of nitrogenous material required

4. Kendall, A. I.: Bacteriology, ed. 3, Philadelphia, Lea & Febiger, 1928, p. 68.

to produce a complete microbe is extremely small, as the following calculation will show:

Bacillus coli is an average-sized organism. It measures approximately 1 micron in diameter and 2 microns in length. Its specific gravity is about 1.030. Its weight, has been shown previously,[5] is approximately 0.000,000,002 mg. Five hundred millions of colon bacilli, therefore, would weigh scarcely 1 mg. About 85 per cent of the total weight of a bacterium is water, hence the substance not water, of 500,000,000 colon bacilli is but about 0.15 mg., an extremely small amount. It follows then that even with a very generous allowance for waste, the quantities of materials required to furnish the structural needs of half a billion fully mature colon bacilli are expressible in small fractions of a gram.

In disproportionate contrast to the minuteness of the average-sized bacterial cell is its capacity to induce extensive chemical changes in its environment. The physiologic basis for this phenomenon lies partly, but not entirely, in the ratio of the surface area of the bacterium to its weight or mass.

The structural needs of living things are, in general, directly related to their mass; their energy requirements, on the other hand, are a function of the ratio of their surface area to their mass. A comparison of these ratios for *B. coli* and for man will be illuminating:

$$\frac{\text{B. coli surface}}{\text{B. coli weight}} : \frac{\text{0.000,01 sq. mg.}}{\text{0.000,000,002 mg.}} \qquad \frac{\text{man surface}}{\text{man weight}} : \frac{\text{1.5 sq. M.}}{\text{70 kilograms}}$$

In the microbe, this ratio of 5,000 to 1 contrasts sharply with the corresponding ratio or relative equality for man. A word of caution must be interjected at this point. The ratio of surface area to weight, while very important, does not explain all the phenomena associated with microbic mass and concomitant microbic chemical change. For example, *B. dysenteriae,* almost exactly the same size as *B. proteus,* induces far less chemical conversion per microbe-milligram hour and per microbe-molecule disruption in mediums from which carbohydrate is excluded, than the latter. This is in keeping with an observation of Theobald Smith [6] that, in general, pathogenic bacteria are chemically less reactive than saprophytic bacteria. In corresponding mediums to which utilizable carbohydrate has been added, these organisms are mutually transformed into producers of lactic acid. Under these conditions their metabolic activities, both qualitatively and quantitatively, approach equality more closely.[7]

5. Footnote 4, p. 26.
6. Smith, Theobald: Am. Med. **8**:711, 1904.
7. Kendall, A. I.: Am. J. M. Sc. **156**:157, 1918.

QUALITATIVE PHENOMENA OF BACTERIAL METABOLISM

From the earliest days of bacteriology, it has been known and recognized that bacteria, each after its kind, "ferment" certain sugars. They fail to "ferment" other sugars. The precision with which these fermentative reactions may be reproduced indicates that a very fundamental phenomenon is involved. Herein lies both a method for the recognition of bacteria through their consistent utilization of certain sugars, and conversely, a method for the recognition of certain sugars through their consistent utilization by certain bacteria.[8] *B. alcaligenes* and a few other microbes do not utilize any known carbohydrates for energy. They are those exceptions in the bacterial family which afford additional evidence of the correctness of the phenomena just mentioned. Another important concept flows from these fermentative reactions of bacteria. Most bacteria produce noteworthy amounts of acidic substances

TABLE 1.—*Products of Action of Various Bacteria on Mediums (1) Containing and (2) Not Containing, Utilizable Sugar*

	Products When Growing in Medium in	
Organism	Presence of Utilizable Sugar	Absence of Utilizable Sugar
B. alcaligenes*	Ammonia; endotoxin	Ammonia; endotoxin
B. coli	Lactic acid	Indol, phenols, ammonia
B. proteus	Lactic acid	Indol, ammonia, soluble proteolytic enzyme
B. welchii	Lactic and butyric acids	Soluble, specific toxin
B. diphtheriae	Lactic acid	Soluble, specific toxin

* B. alcaligenes cannot utilize (ferment) any sugar.

(conspicuous among which is lactic acid) from the utilization of these sugars, whereby they are automatically reduced to a nearly uniform type of chemical activity. Even so, however, their biologic specificity is of course maintained. In absence of utilizable carbohydrate, but under otherwise parallel conditions, each microbe produces from the common nitrogenous constituents of cultural mediums chemical substances which are specific for each kind of organism; otherwise they would be mutually chemically indistinguishable. Stated differently, it appears that the chemical individualities of at least a majority of the commonly known bacteria depend on their utilization of nitrogenous substances for both energy and structural requirements.[7] This is shown qualitatively in table 1. Two highly important facts stand out clearly. In presence of both the usual nitrogenous constituents of cultural mediums and, in addition, a utilizable carbohydrate, the several bacteria cited, with the significant exception of *B. alcaligenes* (which it will be remembered ferments no sugars), produce lactic acid in common. A

8. Kendall and Yoshida: J. Infect. Dis. **32**:355 and 362, 1923.

chemical analysis of these cultural mediums freed from bacteria would therefore afford little or no information of the identity of the microbe in each case, as putrefactive products or substances indicative of the utilization of proteins for energy are entirely absent. This absence of putrefactive products in cultures of bacteria grown in carbohydrate mediums has long been recognized through the observations of Péré,[9] Kruse,[10] Smith,[11] Marshall [12] and many others.

In absence of carbohydrate, but under otherwise parallel conditions, each organism, after its kind, generates distinctive products of growth. *B. coli,* for example, forms indol, phenolic bodies and ammonia; *B. proteus,* indol, ammonia and a powerful, soluble proteolytic enzyme; *B. welchii* and *B. diphtheriae* each a potent, specific soluble toxin. A chemical analysis of these carbohydrate-free mediums, after removal of bacteria, would therefore reveal the identity of the microbe involved. This Dr. Jekyll-Mr. Hyde phenomenon is so generally encountered among bacteria parasitic on or pathogenic for man it may be stated unequivocally that these bacteria, while utilizing carbohydrate for energy, generate in common lactic acid as their characteristic product of metabolism. These same bacteria, utilizing protein derivatives in place of carbohydrate for their energy, produce nitrogenous substances that are chemically specific. The phenomena mentioned refer to conditions under which there is respectively either an excess or an absence of utilizable carbohydrate. If a small quantity of carbohydrate is present in a culture medium, an amount which can be completely fermented by a specified microbe before accumulation of acid restrains its further growth, the initial product of metabolism is, of course, lactic acid. When the sugar is exhausted, the bacteria of necessity turn to the protein ingredients of the medium for their energy requirements. The products of metabolism then include, in addition to lactic acid, gradually increasing amounts of specific, nitrogenous products of metabolism, the chemical composition of which depends on the organism under consideration. Some practical applications of this Dr. Jekyll-Mr. Hyde phenomenon will be discussed later.

QUANTITATIVE PHENOMENA OF BACTERIAL METABOLISM

There is quantitative chemical proof, as well as qualitative chemical evidence, of the correctness of the doctrine that bacteria, as here indicated, utilize carbohydrate in preference to nitrogenous substances

9. Péré: Ann. de Inst. Pasteur **6**:512, 1892.
10. Kruse: Ztschr. f. Hyg. u. Infektionskr. **17**:1, 1894.
11. Smith: J. Exper. Med. **2**:543, 1897.
12. Marshall: J. Hyg. **7**:581, 1907.

for energy, when both are simultaneously available. The chemical analyses recorded in table 2 show the quantitative changes induced in the nitrogenous constituents of cultural mediums by four representative bacteria cultivated both in presence and in absence of dextrose. The methods of analysis [13] and the details of each organism [14] are published elsewhere. The results are expressed in milligrams of nitrogen per hundred cubic centimeters of culture medium.

Table 2 shows comparatively and in detail the nature and the extent of the changes induced by *B. dysenteriae,* Shiga, *B. typhosus* and *B. coli*

TABLE 2.—*Quantitative Changes Induced in Nitrogenous Constituents of Plain Medium and Medium Containing Dextrose by Given Bacteria*

Organism	Nitrogenous Constituents of Medium	Results of Analyses, Mg. per 100 Cc. of Medium								
			Plain Medium				Dextrose Medium			
		Control	1 Day	3 Days	6 Days	10 Days	1 Day	3 Days	6 Days	10 Days
B. dysenteriae, Shiga	Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Protein nitrogen	716	716	758	780	757	716	768	842	790
	Nonprotein nitrogen	284	284	243	221	243	284	231	158	210
	Polypeptid nitrogen	200	213	167	139	158	194	157	74	127
	Aminonitrogen	32	32	27	32	32	27	25	38	38
	Ammonia nitrogen	53	52	48	50	52	54	51	46	46
B. typhosus	Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Protein nitrogen	715	743	754	854	808	733	743	850	827
	Nonprotein nitrogen	285	261	249	146	197	270	261	155	176
	Polypeptid nitrogen	198	163	155	41	91	182	167	62	95
	Aminonitrogen	39	45	41	40	36	41	47	48	37
	Ammonia nitrogen	47	53	54	65	72	45	45	45	45
B. coli	Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
	Protein nitrogen	716	726	790	810	780	726	790	830	800
	Nonprotein nitrogen	284	273	210	189	210	273	211	168	199
	Polypeptid nitrogen	200	190	129	87	106	191	135	87	117
	Aminonitrogen	32	21	11	26	26	30	28	35	37
	Ammonia nitrogen	54	63	69	73	79	53	47	47	47

in the nitrogenous constituents of the cultural mediums, both in presence and in absence of dextrose, and after one, three, six and ten days' incubation at 37 C. These changes are in general progressive up to and including the sixth day, when the cultures have reached their maximal growth. By the tenth day, recessive changes and autolysis have taken place. For brevity, therefore, discussion is limited chiefly to the analyses for the six days' growth.

Metabolism of B. Dysenteriae, Shiga, B. Typhosus and B. Coli.—In Presence of Dextrose: The outstanding and significant feature of the growth of the dysentery, typhoid and colon bacilli, respectively, in the dextrose-containing mediums is an increase in the protein nitrogen frac-

13. Kendall, A. I.: J. Infect. Dis. **30**:211, 1922.

14. On B. dysenteriae Shiga, see Kendall and Haner: J. Infect. Dis. **30**:225, 1922; on B. typhosus, ibid., p. 232; on B. coli, Kendall and Bly: ibid., p. 239.

tion and a concomitant, almost molecule-for-molecule decrease in the polypeptid nitrogen fraction. This is readily seen in table 3. In the dextrose culture of the dysentery bacillus, the protein nitrogen fraction increased 126 mg. in six days, and the polypeptid fraction was concomitantly reduced 126 mg.; in the culture of *B. typhosus,* the gain in protein nitrogen was 135 mg. and the loss in polypeptid nitrogen was 136 mg.; the gain in the protein nitrogen fraction for *B. coli* was 114 mg., corresponding to a loss of 113 mg. for the polypeptid nitrogen fraction. The aminonitrogen and the ammonia nitrogen fractions of the mediums containing dextrose remained practically constant during this six day period of growth. The total nitrogen content also remained unchanged. The explanation for this shifting of polypeptid nitrogen fraction to protein nitrogen fraction, therefore, is very simple and direct.

TABLE 3.—*Bacterial Changes Induced in Nitrogenous Constituents of a Medium Containing Dextrose*

Organism	Nitrogenous Constituent	Results of Analyses, Mg. per 100 Cc. of Medium		
		Control	After 6 Days	Gain or Loss
B. dysenteriae, Shiga	Protein nitrogen....	716	842	+126
	Polypeptid nitrogen	200	74	—126
B. typhosus.....	Protein nitrogen....	715	850	+135
	Polypeptid nitrogen	198	62	—136
B. coli.....	Protein nitrogen....	716	830	+114
	Polypeptid nitrogen	200	87	—113

These organisms, utilizing dextrose for their energy requirements, have multiplied rapidly. Nitrogen in some form is indispensable for the structural needs of bacteria. The bacteria have selected polypeptid nitrogen and woven it molecule for molecule into the complex protein nitrogen of their bodies. For this purpose neither the aminonitrogen nor the ammonia nitrogen fractions of these cultural mediums are as suitable as the ingredients which comprise the polypeptid nitrogen. The aminonitrogen and the ammonia nitrogen fractions therefore remain quantitatively unchanged.[15]

In Absence of Dextrose: In absence of dextrose, when the bacteria must obtain both their energy and structural requirements from the

15. Miss Stephenson (Bacterial Metabolism, London, Longmans, Green & Company, 1930, p. 218) makes the surprising assertion that "ammonia is undoubtedly the chief nitrogenous product of the bacterial decomposition of protein, but it is also the chief nitrogenous food of bacteria: . . . the decrease of ammonia in bacterial cultures on broth, meat, etc., to which carbohydrate has been added, is to be attributed in the main to the increased bacterial multiplication due to an additional and readily available source of carbon."

nitrogenous constituents of the mediums, the course of events is somewhat different, as is clearly indicated in table 4.

Here again, there is a very material increase in the protein nitrogen fractions, indicative of the increase in the bacterial population after six days' growth. With the exception of *B. dysenteriae,* this increase in protein nitrogen is more than compensated for by losses in the polypeptid fraction. In other words, the polypeptid loss exceeds the gain in protein nitrogen. The excess loss in the nonprotein fraction is found chiefly in the increase of the ammonia fraction of the cultural mediums. This is deamination. It is a measure of the amounts of nitrogenous constituents of the mediums used by the bacteria for their energy requirements. Ammonia as such has no energy value for microbic growth.[16] It is eliminated from the peptid molecules prior to their oxidation for the energy needs of the microbes, and it consequently collects as ammonia in the cultures. This is shown very clearly by the

TABLE 4.—*Changes Induced in Nitrogenous Constituents of Plain Medium by Given Bacteria*

Organism	Gain or Loss on Six Days' Incubation, Mg. per 100 Cc. of Medium			
	Protein Nitrogen	Polypeptid Nitrogen	Amino-nitrogen	Ammonia nitrogen
B. dysenteriae, Shiga.....	+ 64	— 61	0	— 3
B. typhosus.....	+139	—157	+ 1	+ 18
B. coli.....	+ 94	—113	— 6	+ 19

analyses. It may be remarked parenthetically here that there is no accumulation of ammonia in those cultures in which dextrose is available as a source of energy. Returning to *B. dysenteriae*: it will be noted that the protein nitrogen increase in the culture of this organism is associated with an almost identically equivalent loss in polypeptid nitrogen. In this respect, the dysentery bacillus appears to differ chemically in its metabolism from *B. typhosus* and *B. coli.* It will be recalled, however, that *B. dysenteriae* (Shiga variety), unlike most other parasitic and pathogenic bacteria, can utilize the carbohydrate moiety of the protein molecule[17] for its energy requirements. Hence the dysentery bacillus is using carbohydrate for energy, even in dextrose-free mediums, and its metabolism here should, and does, approach that of a culture of this microbe in a medium containing dextrose.

Comment on Metabolism of *B. dysenteriae,* Shiga, *B. typhosus* and *B. coli*: These studies of metabolism in sugar-free mediums and in mediums containing dextrose reveal one important common feature:

16. Except for certain types of soil bacteria. Cf. Waksman: J. Bact. **7**:231, 1922.

17. Kendall and Farmer: J. Biol. Chem. **12**:13 and 215, 1912.

in each instance the polypeptid fraction of the medium is by far the most readily utilized, both for the structural and (in absence of dextrose) for the energy needs of the organisms herein described. The totality of discernible nitrogenous change in the mediums containing sugar is a molecule-for-molecule loss of polypeptid nitrogen for a corresponding gain in protein nitrogen. In the sugar-free mediums, with the exception of *B. dysenteriae,* for reasons previously mentioned, the polypeptid fraction is used not only for the structural needs of the newly formed bacteria as indicated by the gain in protein nitrogen, but also for their energy needs. This is shown by the reciprocal loss of polypeptid nitrogen and the concomitant gain by deamination (ammonia nitrogen). These observations are in harmony with the well attested fact that a great majority of bacteria intimately associated with man and the animals grow far better in mediums containing peptones, which are relatively rich in polypeptid nitrogen, than they do in mediums containing either single amino-acids (and therefore rich in aminonitrogen) or in mediums that have ammonium salts as sources of nitrogen. The latter are, of course, rich in ammonia nitrogen.

Metabolism of B. Proteus.—B. Proteus differs from the bacteria just described in that it is a much more vigorously growing microbe [18] and also in that it produces, in absence of utilizable carbohydrate, a very active soluble proteolytic enzyme. This enzyme brings about deep-seated changes in the nitrogenous constituents of cultural mediums; hence for relative completeness, the metabolism of *B. proteus* is considered here. The experiments with *B. proteus* were of two kinds (1) cultivation of *B. proteus* in mediums containing graded amounts of dextrose, from 0 to 1.5 per cent, and (2) the addition of sterile filtrates of these cultures to sterile gelatin in the proportion of 1 part culture filtrate freed from bacteria to 19 parts sterile gelatin. Three days' incubation of these mixtures of culture filtrates and gelatin was allowed, to permit maximum change before the chemical analyses were made. One half of table 5 contains the details of the distribution of nitrogen in the cultures at the start and after twenty-four hours', three, five and seven days' incubation at 37 C.

Table 6 taken from the first half of table 5 shows the changes expressed in milligrams per hundred cubic centimeters, which *B. proteus* induces in the total nitrogen fraction of the several mediums, in presence and in absence of varying amounts of dextrose. The analyses are those of the first, third, fifth and seventh days of incubation.

18. For a description of the B. proteus group, see Kendall, Cheetham and Hamilton: J. Infect. Dis. **30**:251, 1922.

Table 6 shows clearly that the protein nitrogen fraction diminishes steadily and rapidly, although the retarding effect of 0.75 per cent dextrose is noticeable, both in time and in amount. This loss in the

TABLE 5.—*Nitrogenous Metabolism of B. Proteus and of Its Soluble, Proteolytic Enzyme*

		Results of Analyses, Mg. per 100 Cc.											
		B. Proteus, Culture						B. Proteus, Soluble Enzyme					
				Dextrose						Dextrose			
Nitrogenous Constituent	Day	Control	Plain	0.1 %	0.5 %	0.75 %	1.5 %	Control	Plain	0.1 %	0.5 %	0.75 %	1.5 %
Total	1	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein		721	531	564	676	721	743	803	482	420	639	703	795
Nonprotein		280	470	437	325	280	258	195	514	588	369	300	205
Polypeptid		209	389	361	253	213	191	151	485	540	325	258	161
Aminonitrogen		39	32	28	32	29	30	30	37	36	35	35	32
Ammonia		32	49	48	40	38	37	12	12	12	10	10	12
Total	3	1,000	1,000	1,000	1,000	1,000		1,000	1,000	1,000	1,000	1,000	
Protein		721	239	239	453	531		803	348	168	436	520	
Nonprotein		280	762	762	548	470		195	648	840	571	488	
Polypeptid		209	647	647	469	400		151	568	747	516	441	
Aminonitrogen		39	36	36	30	28		30	66	78	45	44	
Ammonia		32	89	89	49	42		12	13	15	12	12	
Total	5	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein		721	161	147	239	340	822	796	244	247	410	509	788
Nonprotein		280	840	847	762	661	179	202	752	752	587	499	211
Polypeptid		209	663	664	648	567	103	158	687	682	526	433	168
Aminonitrogen		39	33	31	35	30	39	30	50	52	49	50	30
Ammonia		32	144	149	79	64	37	13	15	14	14	14	13
Total	7	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein		721	38	94	94	105	732	796	240	293	393	393	740
Nonprotein		280	963	907	907	896	269	202	752	706	604	604	260
Polypeptid		209	723	687	730	722	185	150	688	624	530	531	210
Aminonitrogen		39	36	25	40	33	44	36	55	60	56	55	33
Ammonia		33	204	195	137	141	39	19	16	20	19	19	19

TABLE 6.—*Changes Which B. Proteus Induces in Total Nitrogen Fraction of Plain Medium and Mediums Containing Dextrose*

	Results of Analyses, Mg. per 100 Cc.					
Days	Control	Plain	0.1 Dextrose	0.5 Dextrose	0.75 Dextrose	1.5 Dextrose
1	721	531	564	676	721	743
3	721	239	239	453	531	...
5	721	161	147	239	340	822
7	721	38	94	94	105	732

protein nitrogen is caused by the activity of the proteolytic enzyme, and as it occurs in spite of the fact that bacteria are multiplying rapidly, it is an indication of the potency of the enzyme. The rapid decrease of protein nitrogen shown in the second half of table 5, where the enzyme acting alone is studied, is another means of estimating its potency. Coincident with the decrease in protein nitrogen, there is a corresponding, but greater, loss in the polypeptid fraction (first half of table 5), with the exception of the medium having 1.5 per cent dextrose. Here the protein fraction increases from 22 mg.

the first day of incubation, to a maximum of 101 mg. the fifth day, when the culture seems to be at its greatest luxuriance. The polypeptid nitrogen of the 1.5 per cent dextrose culture decreases with the gain in protein nitrogen and almost exactly molecule for molecule. This is in accord with the observations made on *B. dysenteriae, B. typhosus* and *B. coli* in mediums containing dextrose. It is an expression, not only of the building of new bacterial bodies, but also (when considered in connection with the corresponding figures for the mediums having less dextrose) of the fact that 1.5 per cent dextrose is a greater amount than this strain of *B. proteus* can utilize before the accumulation of acids from fermentation arrest its growth. Stated differently, *B. proteus* can, and does elaborate a soluble, proteolytic enzyme in mediums containing up to 0.75 per cent dextrose. The greater the amount of dextrose up to this amount, the slower is the appearance of the enzyme.

TABLE 7.—*Changes in Ammonia Nitrogen Induced by B. Proteus in Plain Medium and in Mediums Containing Dextrose*

	Results of Analyses for Ammonia Nitrogen, Mg.					
Days	Control	Plain	0.1 Dextrose	0.5 Dextrose	0.75 Dextrose	1.5 Dextrose
1	32	49	48	40	38	37
3	32	59	89	49	42	..
5	32	144	149	79	64	37
7	33	204	195	137	141	39

Turning to the other nitrogenous changes: it is apparent first of all that the aminonitrogen fraction varies but little during the first seven days of growth; that is, during the period when the cultures are most vigorous. In fact, the maximum change noted, a decrease of from 39 to 25 mg. (seven day culture in .0.1 per cent dextrose), is registered in presence of a decrease in protein nitrogen from 721 mg. to 94 mg.—in other words, a loss of 627 mg. This disproportion between the loss in protein nitrogen and the concomitant insignificant change in aminonitrogen seems to explain the negative results obtained by Berman and Rettger [19] and others who have relied on the formaldehyde (Sörensen) titration method in their study of the effects of utilizable sugars on bacterial metabolism.[20]

Aside from the rapid degradation of the protein nitrogen, the most striking alteration in the nitrogenous constituents is the increase in ammonia nitrogen. The respective amounts for the first week of incubation are as shown in table 7.

19. Berman and Rettger: J. Bact. **3**:389, 1918.
20. Cf. also Jones: J. Infect. Dis. **27**:169, 1920.

The increase of ammonia nitrogen in the plain and 0.1 dextrose mediums is rapid, and at the end of seven days is very great indeed. The effects of increasing amounts of dextrose are very clearly shown. Even 0.75 per cent of this sugar, in presence of considerable fermentation acid produced by its utilization, failed to restrain the action of the bacilli on the nitrogenous constituents when the dextrose was used up. On the other hand, in presence of sufficient dextrose (1.5 per cent) deamination did not occur.

It would appear from this, that other observers, who state that formation of ammonia (deamination) does not occur in presence of dextrose because of the accumulation of acid, are in error: the accumulation of acid, is, of course, an indication that the bacteria are utilizing the sugar for energy; otherwise there would be no acid. The continuance of the formation of acid is measured by the residuum of sugar. If the amount of sugar is sufficient to supply the microbes until

TABLE 8.—*Nitrogenous Metabolism of the Soluble Enzyme of B. Proteus in Plain Medium and in Medium Containing Dextrose*

Nitrogenous Constituent	Gain or Loss on Five Days' Incubation, Mg. per 100 Cc. of Medium				
	Plain	0.1 Dextrose	0.5 Dextrose	0.75 Dextrose	1.5 Dextrose
Protein nitrogen	−532	−547	−386	−206	− 8
Polypeptid nitrogen	+529	+522	+368	+275	+10
Aminonitrogen	+ 20	+ 22	+ 19	+ 20	0
Ammonia nitrogen	+ 2	+ 1	+ 1	+ 1	0

they have formed enough acid from its fermentation to inhibit their further activity, the protein ingredients of the medium are not changed to any great extent, other than to build up the bodies of bacteria as hitherto stated. If, however, the sugar is exhausted before sufficient acid is generated from it to inhibit further activity, the nitrogenous ingredients are then of necessity utilized for energy, and products indicative of this utilization of protein accumulate. This is shown very clearly in the first half of table 5.

The second half of table 5 contains the analytical data concerning the action of the soluble bacteria-free enzyme of *B. proteus* on sterile (carbol) gelatin. The several samples were taken at the time when the various mediums were analyzed.

The two most striking facts brought out by the analytical figures for the actions of the enzyme are (1) the reduction of the protein nitrogen fraction, in all except the medium having 1.5 per cent of dextrose, with an almost exactly concomitant increase, in each instance, of polypeptid nitrogen, and (2) the absolutely fixed ammonia nitrogen, which does not in any instance vary more than 2 mg. from the controls. Table 8, showing the losses in protein nitrogen, the gains in polypeptid

nitrogen and the consistent values for aminonitrogen and especially for ammonia nitrogen, will make this statement clear. The amounts are recorded for the fifth day of incubation only: at this time the enzymatic action was apparently maximal.

It will be seen that the action of the soluble, proteolytic enzyme of *B. proteus* is not restrained even by 0.75 per cent dextrose, since the loss of protein nitrogen in the experiment resulted in a total decrease of 296 mg. of protein nitrogen from a total of 796 mg., or about 37 per cent. At the same time, however, this amount of dextrose (0.75 per cent) retarded and restricted the formation of enzyme markedly. This is shown in the corresponding loss in the medium having but 0.1 per cent dextrose, in which the protein nitrogen decreased from 796 mg. to 244 mg., a loss of 552 mg., or 68 per cent. The medium having 1.5 per cent dextrose showed a loss of but 8 mg. of protein nitrogen from a total of 796 mg., which is about the limit of precision of the method.

Turning to changes in aminonitrogen: it is noted that the amount found in mixtures of plain medium and enzyme and of enzyme and medium containing 0.1 per cent dextrose shows an increase of about 20 mg., in contrast with a loss in protein nitrogen, of some 550 mg. This is reduced to zero in the mixture of enzyme and broth containing 1.5 per cent dextrose. It appears that this soluble enzyme of *B. proteus,* in reducing the protein constituents of the medium, has a deep-seated action, reminiscent in this respect of trypsin rather than of pepsin.[21]

Most significant of all the data relating to *B. proteus* are those for ammonia nitrogen. Five day cultures of *B. proteus,* except in medium containing 1.5 per cent dextrose, showed increments of ammonia ranging from about 100 mg. in the medium having 0.50 and 0.75 per cent dextrose, to nearly 200 mg. in the medium having either no dextrose or 0.1 per cent (first half of table 5). This increase in ammonia is due to the deamination which precedes the utilization of the nitrogenous constituents of the medium for energy.[22] It will be recalled that there was an increase of but 6 mg. in the corresponding medium having 1.5 per cent dextrose. The sterile filtrates of these several cultures, which, with the exception of the one of the culture in medium containing 1.5 per cent dextrose, have a very strongly proteolytic enzyme, do not in any instance increase the ammonia nitrogen more than 2 mg. (second half of table 5). The striking difference between the action

21. For a comparison of the enzyme of B. proteus with pepsin and trypsin, see Kendall and Keith: J. Infect. Dis. **38**:193, 1926.

22. Cf. also Sears: Thesis, Stanford University, January, 1916.

of the living organisms plus enzyme in culture and the enzyme without living organisms in the mixture of sterile gelatin and culture filtrate, shows conclusively, so it seems, that deamination is brought about by living bacteria. It is a step preparatory to the utilization of nitrogenous constituents of cultural mediums for energy. The absence of ammonia from corresponding cultures containing sufficient dextrose is additional evidence: here the bacteria derive their energy from the carbohydrate, utilizing the nitrogenous constituents chiefly for their structural needs. Ammonia, therefore, as such, is a waste product, not a structural element.[23]

SIGNIFICANCE OF BACTERIAL METABOLISM

In the Study of Intestinal Bacteriology.—It is a well attested fact, known since Escherich's classic studies on the subject,[24] that the bacterial intestinal flora of the normal nursling is essentially fermentative: proteolytic bacteria and proteolysis alike are absent. As the infant becomes older and the diet more varied, the character of this intestinal flora undergoes a marked change: the fermentative organisms, *B. bifidus, B. acidophilus* and *Micrococcus ovalis,* tend to disappear, and organisms, as *B. coli,* that can accommodate their metabolism to presence or absence of carbohydrate take their place. That this microbic change parallels the dietary change appears to be rather generally agreed on, but few observations are recorded which give a fairly definite picture of the comparative chemical changes induced by intestinal bacteria of nurslings, artificially fed babies, adolescents and adults. Table 9, abbreviated from tables previously published,[25] shows the changes induced in the nitrogenous constituents of cultural mediums, similar to those already studied in the preceding tables, when they are respectively inoculated with the mixed intestinal bacteria from a normal nursling, a normal artificially fed infant, a normal adult and an adult having a heavy intestinal overgrowth of *B. welchii.*

The salient analytical details are shown for the normal nursling, the artificially fed infant, the normal adult and the adult with gas bacillus overgrowth. It will be seen that the conditions of experiment here are in a measure the reverse of a feeding experiment in man in that it is the mixed intestinal bacteria that are fed on selected diets.

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23. See Sears and Gourley: J. Bact. **15**:357, 1928.

24. Escherich: Darmbakterien des Saüglings, Stuttgart, 1886.

25. Kendall; Day, and Walker: Chemistry of the Intestinal Flora of Nurslings, J. Infect. Dis. **38**:200, 1926; Chemistry of the Intestinal Bacteria of Artificially Fed Infants, ibid., p. 205; Chemistry of the Intestinal Flora of Normal Adults, ibid., p. 211; Chemistry of the Intestinal Flora of Man Containing Abnormal Numbers of Gas Bacilli, ibid., p. 217.

It must be borne in mind in these experiments with the intestinal flora, that a considerable variety of types of bacteria are, or may be, present in each inoculum. It follows that the chemical changes elicited by the growth of these mixtures of bacteria connote a resultant totality of reaction which may be greater in amount than, and even different in character from, the sum of the activities of the several kinds of bacteria growing in pure culture.[26] This has been shown, for example, in the associative action of certain strains of *B. coli* and *B. mesentericus*.[27] Growing separately in pure culture in milk, *B. coli* causes coagulation, and *B. mesentericus*, digestion. Grown symbiotically, they induce a stormy fermentation closely simulating that of *B. welchii*. Nevertheless, as is clearly shown in table 8, the several distinct types of intestinal flora, approach, both qualitatively and quantitatively, one or another of the types of chemical activity described in previous sections. The origin and significance of these several manifestations of chemical activity have been explained at length; therefore, only the more general phenomena will be discussed in association with the respective types of intestinal flora here.

Normal Nursling (table 9): The mixed intestinal flora from the normal breast-fed infant is characteristically fermentative. There is complete absence of putrefactive bacteria, and there is no evidence of proteolytic activity. In the sugar-free medium, however, there is slight deamination, as compared with the corresponding growth in the dextrose medium. The small amount of ammonia produced is indicative of a preponderance of *M. ovalis*, rather than of *B. acidophilus* or of *B. bifidus* in the culture. *M. ovalis* grows better in mediums freed from carbohydrates than either of the bacilli.[28] Smears made from the plain and the dextrose mediums show few *acidophili* and *bifidi* in the former, but many *bifidi* in the latter. *M. ovalis* is predominant in the former and abundant in the latter. The increase in protein nitrogen, with a concomitant decrease in polypeptid nitrogen, most marked on the sixth day of incubation, is in accord with previous observations. The significance of the obligately fermentative and lactacidogenic intestinal flora, the natural defensive barrier of the breast-fed infant against microbic intestinal infectants, has been recognized ever since Escherich's classic study of the bacteria of nurslings.[24]

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26. Castellani and Chalmers: Brit. M. J. **2**:855, 1917; **1**:183, 1919. Holman: J. Infect. Dis. **39**:145, 1926. Sears and Putnam: ibid. **32**:270, 1923. Kendall (footnote 4, p. 78).

27. Kendall: Boston M. & S. J. **163**:322, 1910.

28. Kendall and Haner: Metabolism of Micrococcus Ovalis, B. Acidophilus, B. Bifidus, J. Infect. Dis. **35**:67, 77 and 89, 1924.

Normal Artificially Fed Infants: The outstanding chemical feature of the metabolism of the intestinal flora of the artificially fed infant lies in the striking contrast between the considerable nitrogenous changes induced by the mixed flora in the sugar-free medium and the very small nitrogenous changes induced in the corresponding sugar-containing

TABLE 9.—*Changes Induced in the Nitrogenous Constituents of Cultural Mediums, When These Are Inoculated with the Mixed Intestinal Bacteria from Normal Nursling, Normal Artficially Fed Infant, Normal Adult and Adult with Overgrowth of Gas Bacillus*

	Results of Analyses, Mg. per 100 Cc. of Medium									
	Plain Medium					Dextrose Medium				
Nitrogenous Constituent	Control	1 Day	3 Days	6 Days	9 Days	Control	1 Day	3 Days	6 Days	9 Days
Normal nursling										
Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen	770	780	770	790	770	790	790	790	800	792
Nonprotein nitrogen	230	220	230	207	231	210	210	210	200	208
Polypeptid nitrogen	173	155	156	120	134	152	151	153	138	150
Aminonitrogen	36	32	32	38	41	35	30	28	34	34
Ammonia nitrogen	21	33	42	47	55	22	27	26	24	22
Artificially fed infant										
Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen	770	760	690	680	675	770	780	760	760	770
Nonprotein nitrogen	229	240	310	321	324	229	219	240	240	230
Polypeptid nitrogen	182	168	213	179	164	182	169	184	186	163
Aminonitrogen	33	39	20	22	29	33	35	32	32	42
Ammonia nitrogen	14	33	89	123	131	14	16	15	15	23
Normal adult										
Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen	816	803	796	792	778	828	828	820	829	828
Nonprotein nitrogen	183	195	204	206	216	171	182	182	171	171
Polypeptid nitrogen	131	131	127	83	72	121	129	127	113	111
Aminonitrogen	30	35	30	31	36	30	30	30	32	34
Ammonia nitrogen	21	28	48	91	109	20	24	26	26	26
Adult with excessive number of gas bacilli										
Total nitrogen	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Protein nitrogen	816	791	604	515	450	816	816	805	805	828
Nonprotein nitrogen	182	208	390	488	550	183	183	195	195	171
Polypeptid nitrogen	124	126	220	226	252	129	143	155	155	113
Aminonitrogen	38	44	37	43	25	33	19	18	16	34
Ammonia nitrogen	20	37	134	219	270	20	22	23	24	24

medium. In the former, a reduction of the protein nitrogen fraction of about 90 mg. and of aminonitrogen of 10 mg. is rather more than compensated for by an increase of ammonia nitrogen amounting in the aggregate to 109 mg. It is clear that this shifting of nitrogenous constituents is not due to a proteolytic enzyme, else the polypeptid nitrogen would increase proportionally. The organism responsible for this type of reaction is a strong deaminizer. It appears to be quite similar to that described in another study (organism 62).[29] In the corresponding dextrose medium, the nitrogenous changes are very small indeed: there

29. Kendall; Day; Walker, and Haner: Bacteriology and Chemistry of Adult Duodenal Contents, J. Infect. Dis. **40**:677, 1927.

is no evidence of proteolysis or of putrefaction. In this respect the chemical change is reminiscent of that induced by the intestinal flora of the normal nursling. The effects of dextrose in reducing the action of the bacteria on the nitrogenous constituents of the medium for energy needs are clearly shown. There is no evidence of deamination, proteolysis or putrefaction. The very small nitrogenous changes are those attributable to the usual building up of polypeptid nitrogen to protein nitrogen in the bodies of the growing bacteria.

Normal Adult: The changes induced by the mixed intestinal flora of the normal adult are not very different from those just described for the artificially fed baby. There is evidence of some proteolysis in the sugar-free medium. This is indicated by a small decrease in the protein nitrogen fraction and also by much deamination. The gain in ammonia nitrogen is some 70 mg.; it is less than that observed in the sugar-free cultivation of the mixed flora of the artificially fed infant, but materially greater than that characteristic of the normal nursling. In presence of dextrose, the chemistry of the flora of the normal adult reverts to the typical fermentative pattern characteristic of the several floras just described, as well as of those of the dysentery, typhoid, colon and *proteus* bacilli. There is no significant deamination or evidence of proteolysis. The significant nitrogenous change is a transfer of polypeptid nitrogen to protein nitrogen, consonant with the growth of bacteria in the medium.

Adults with Excessive Number of Gas Bacilli: The evidence educed thus far from the chemical study of the mixed intestinal floras of nurslings, babies fed by bottle and normal adults has brought to light two principal facts. In the first place, in presence of carbohydrate, all these bacteria are reduced chemically to one common level—that of producers of lactic acid—in which state the bacteria have minimal action on the nitrogenous constituents of these mediums. In the second place, the mixed intestinal flora of the breast-fed infant is nonproteolytic and nonputrefactive, even in absence of utilizable carbohydrate, whereas the floras of the artificially nourished infant and of the normal adults, in absence of utilizable carbohydrate, are more or less proteolytic and putrefactive, as shown by the concomitant changes in the nitrogenous constituents of these cultural mediums. For purposes of comparison, the chemistry of mixed intestinal bacteria from an adult with an overgrowth of gas bacilli in the intestinal tract is introduced at this point. The salient details appear in table 9. The most striking chemical change is found in the ammonia fraction, which by the ninth day has increased to the surprising amount of 250 mg. This is accompanied by a decrease of 366 mg. in the protein nitrogen, indicating active proteolysis, and a gain of 128 mg. of polypeptid nitrogen, which, with the gain in

ammonia, accounts for almost the entire loss in protein nitrogen. It is evident, from what is known of the chemistry of *B. welchii* (the gas bacillus) [30] that the proteolytic changes are not due to the Welch bacillus, but to associated bacteria, among which nonfermenters of dextrose, as *B. alcaligenes* and the one found in the flora of the artificially fed baby, are usually prominent. Here again is an example of symbiotic action, in which two bacteria, working together, produce unusual chemical changes. In sharpest contrast with the deamination found in the sugar-free medium is the utter absence of more than minimal nitrogenous changes in the corresponding dextrose-containing culture. Here there is the customary increase in protein nitrogen with its concomitant decrease in polypeptid nitrogen. Deamination is reduced almost to zero. A word of caution must be injected here, however. The addition of carbohydrate to the diet of the patient whose fecal bacteria are under consideration would be very harmful. If this were done, the gas bacilli would go on a rampage, with consequences very distressing to the victim of such ill-advised dietary treatment. Well soured milk is indicated in such cases. The gas bacilli are thereby frustrated in their growth, and the patient is benefited.[31] The results, in terms of chemical change on a diet rich in sour milk, would be very similar to those achieved by the addition of dextrose to the culture medium. Deamination and indications of proteolysis would be reduced to the vanishing point, while at the same time the activity of the gas bacilli would be curbed.

In Clinical Practice.—Several important clinical applications of this sparing action of carbohydrates for nitrogenous substances readily suggest themselves, as follows:

Reduction of Intestinal Putrefaction: It is generally believed that urinary indican is an indication of intestinal putrefactive processes of microbic causation. This is in the main true. However, a much more important substance is overlooked in the ordinary tests, namely, indol. Indoluria, not indicanuria, has the greater clinical significance, even though both substances have a common point of origin in intestinal putrefaction. Under ordinary circumstances, indol is oxidized in the liver to indoxyl, then paired, and excreted as indoxyl sodium sulphonate. This is urinary indican. If, however, this function of the liver is interfered with and oxidation of indol does not take place, indol, not indican, many appear in the urine. This may also happen if the amount of indol

30. Kendall; Day, and Walker: J. Infect. Dis. **30**:141, 1922.

31. For clinical and chemical aspects of gas bacillus overgrowth in the intestinal tract of man, see Kendall: Intestinal Intolerance for Carbohydrate Associated with Overgrowth of Gas Bacillus (Bacillus Welchii), J. A. M. A. **86**:737, 1926; Kendall and Varney: J. Infect. Dis. **41**:143, 1927; Kendall and Gebauer: ibid. **47**:261, 1930.

is temporarily greater than the liver can manage. Indoluria, therefore, is, or may be, an indication of impaired function of the liver, as well as of intestinal putrefaction. The formation of indol in the urine, as has been indicated, may be greatly restricted by suitable dietary procedure.

Dietary Treatment in Typhoid Fever and Dysentery: The effects of diets high in carbohydrate in typhoid fever were studied by Coleman and Shaffer [32] from a purely chemical point of view. They found that the loss of weight was materially reduced, and the general condition of the patient materially improved when large daily feedings of lactose were given, and were disposed to attribute these gains to the well known dictum that "carbohydrate spares body protein." An additional and highly important factor seems to have been overlooked, however. The diet high in lactose has a profound effect on the metabolism of the typhoid bacillus itself. In presence of carbohydrate this microbe, of course, becomes a producer of lactic acid. Herein, probably lies the otherwise unexplainable reduction in toxemia which Coleman and Shaffer noticed when their patients were placed on the diet high in calories. Under these circumstances the typhoid bacilli, in virtue of their altered metabolism (Dr. Jekyll and Mr. Hyde again), utilize carbohydrate for energy in preference to protein. The first deliberate attempts to utilize the sparing action of carbohydrates for proteins with its associated change in the products of metabolism in the treatment of disease were made in Boston in 1911 and subsequent years.[33] The results were satisfactory in cases of bacillary dysentery and in gas bacillus diarrheas. The same procedure was subsequently used by Torrey [34] in his studies on the effects of diet in typhoid fever. The importance of these dietary adjuvants in the field of clinical medicine is gradually being recognized.[35]

Production of Soluble Specific Toxins by Bacteria: An important example of the sparing action of carbohydrate for protein, which is an essential factor in the commercial production of specific antitoxin, was first described by Theobald Smith [36] many years ago, before much was known of the chemistry of bacteria. He found that diphtheria bacilli do not yield toxin in presence of more than minimal amounts of

32. Coleman and Shaffer: Protein Metabolism in Typhoid Fever, Arch. Int. Med. **4**:538, 1909.

33. Kendall: Boston M. & S. J. **164**:288, 1911; Kendall and Smith: ibid., p. 306.

34. Torrey: J. Infect. Dis. **16**:72, 1915.

35. Stokes, J. H.: The Effect on the Skin of Emotional and Nervous State: III. Theoretical and Practical Consideration of a Gastro-Intestinal Mechanism, Arch. Dermat. & Syph. **22**:962, 1930.

36. Smith, Theobald: J. Exper. Med. **4**:373, 1899.

dextrose. It is now known that in presence of dextrose the diphtheria bacillus forms lactic acid in place of toxin.[37] As potent toxin is essential for the production of antitoxin, this principle of reducing utilizable sugar in mediums for the production of toxin is rigorously adhered to. It should be stated that the same phenomenon is met with in the preparation of the toxin of *B. welchii* [38] and others.

"Resting Bacteria" in Vaccine Therapy: A word should be said at this time about the newly explored field of "resting bacteria." The significant pioneer work was done largely by Quastel [39] and his associates, between the years 1924 and 1928. He investigated several problems of significance to the understanding of microbic action by means of these "resting" organisms.

"Resting bacteria" are actively growing organisms harvested at the time at which they are proliferating most actively. After careful washing by centrifugation, repeated several times to insure removal of all alien material, they are kept in suspension in salt solution at the temperature of the icebox for several days without material impairment of their original activities. These "resting" bacteria "initiate changes in substrates, which they, as proliferating bacteria, would subsequently use for their energy requirements."[40] The amount of chemical changes such bacteria can bring about in a very few hours is remarkable.[41] One very suggestive clinical application of "resting" bacteria has arisen in connection with vaccine therapy. It has been found [42] that bacteria harvested as described and therefore freed from adhering cultural medium, etc., when killed by exposure to tenth-normal formaldehyde solution (neutral in reaction) for two hours, are much more effective in the treatment of furuncles and similar focal infections. There is also considerable evidence that pneumococci, prepared in similar manner after growth on blood agar, have materially greater antigenic and protective power than can be had from cultures of the same types not carefully washed from adherent products of growth and constituents of cultural medium. This aspect of bacteriology is still very immature, but it holds forth promise of many important contributions which will help fill in some of the great gaps in present knowledge of the mechanisms of growth in general and of microbic infection in particular.

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37. For chemical evidence, see Kendall; Day, and Walker: J. Am. Chem. Soc. **35**:1210, 1913; **36**:1950, 1914.

38. Bull and Pritchett: J. Exper. Med. **26**:603 and 687, 1917.

39. Quastel: Biochem J., 1924-1927, vols. 18-21; for brief summary, see J. Hyg. **28**:139, 1928.

40. Kendall and Ishikawa: J. Infect. Dis. **44**:282, 1929.

41. Kendall; Ishikawa, and Friedemann: J. Infect. Dis. **47**:186, 1930.

42. Details will be published soon.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Stanhope Bayne-Jones, professor of bacteriology in the school of medicine and dentistry of the University of Rochester, has been appointed master of college and professor of bacteriology in the school of medicine of Yale University.

Kenneth M. Lynch, professor of pathology in the Medical College of the State of South Carolina, has been given the honorary degree of LL.D. by the University of South Carolina.

Carroll Gideon Bull, professor of immunology in the school of hygiene and public health of the Johns Hopkins University, has died.

Charles Phillips, has resigned as professor of pathology in the Medical College of Virginia in Richmond to assume the directorship of the laboratories of the Scott and White Clinic, Temple, Texas.

Herbert H. Waite, professor of bacteriology at the University of Nebraska, Lincoln, has died at the age of 62.

Ralph McBurney has been appointed professor of bacteriology and hygiene in the University of Alabama.

Axel Holst, professor of bacteriology and hygiene in the University of Oslo and well known for his pioneer investigations in deficiency diseases, has died at the age of 70.

Emmerich von Haam, associate professor of pathology in the school of medicine in the University of Arkansas, has been appointed assistant professor of pathology and bacteriology in the State of Louisiana Medical Center, New Orleans.

Shibamiro Kitasato, celebrated pioneer Japanese bacteriologist, has died at the age of 72. He was a pupil of Robert Koch. He first grew the tetanus bacillus in pure culture; he was the co-discoverer with Behring of diphtheria and tetanus antitoxins in 1890; in 1894 he discovered the plaque bacillus at about the time that it was discovered independently by Yersin; he was director of the Imperial Institute for Infectious Diseases of Japan before he organized the Kitasato Institute for Infectious Diseases in Tokyo.

Alvin G. Foord, pathologist in the Buffalo General Hospital, has been appointed pathologist in the Pasadena Hospital, Pasadena, Calif.

Society News.—At the recent meeting of the American Medical Association in Philadelphia gold medals were awarded to Jacob Furth of the Henry Phipps Institute of the University of Pennsylvania, for his work on experimental leukemia in the mouse and the fowl, and to J. Parsons Schaeffer and Warren B. Davis, Jefferson Medical College, for anatomic studies on the nasal sinuses. Silver medals were awarded to Bedford Shelmire, Dallas, Texas, and to W. E. Dove, U. S. Bureau of Entomology for their work on the spread of typhus fever by the tropical rat mite, and to Harrison S. Martland, A. O. St. George, A. O. Gettler and R. H. Mueller for demonstration of industrial radium poisoning.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

OSSIFICATION: I. CALLUS FORMATION AND CALCIFICATION. I. NEWTON KUGELMASS and RICHARD N. BERG, Am. J. Dis. Child. **41**:236, 1931.

The relation between callus formation and the amount and rate of calcification was studied in rabbits. The greater the local formation of fibrous tissue, the greater was the amount and degree of calcification, all other conditions remaining the same. The blood calcium tends to be higher and the phosphate lower in the course of normal bone repair, and the more rapid the rate of calcification the more marked is this alteration in the calcium and phosphate content of the blood. Injection of an alkaline solution of trypsin, at the site of fracture to produce fibrous dissolution, markedly retarded the union of bones in comparison with that in the control series. Injection of a solution of tissue fibrinogen at the site of fracture, to stimulate increased callus formation, markedly accelerated the union of bones in comparison with that in the control series. The maintenance of animals with induced fractures on a diet high in protein, to increase the blood-clotting factors, produced no local effect in bone repair as compared with the control series. Ossification is a local transformation of fibrous tissue into bone, the constituents of which are derived from the blood. Bone repair is a local rather than a systemic process, and union may therefore be induced or accelerated by local measures.

AUTHORS' SUMMARY.

MONGOLISM IN ONE OF TWINS. J. I. WARING, Am. J. Dis. Child. **41**:351, 1931.

This case of mongolism in one of dizygotic twins supports the view that the condition is the result of a defect in germ plasm and not due to the general physical condition of the parents.

AUTHOR'S SUMMARY.

SPLENOMEGALY AND HEPATIC ENLARGEMENT IN HEREDITARY HEMORRHAGIC TELANGIECTASIA. THOMAS FITZ-HUGH, JR., Am. J. M. Sc. **181**:261, 1931.

Four cases of hereditary hemorrhagic telangiectasis are reported, which exhibited the following noteworthy features: splenomegaly and hepatic enlargement; increasing intolerance to transfusions of blood, resulting in posttransfusion jaundice (and death in two); the coincidence of identical blood group (0) in all of the patients tested who presented the splenomegalic syndrome.

AUTHOR'S SUMMARY.

HYPERTENSION IN CASES OF CONGENITAL POLYCYSTIC KIDNEY. FREDERICK W. SCHACHT, Arch. Int. Med. **47**:500, 1931.

Evidence at hand indicates that in the majority of cases of polycystic kidney, significant or persistent hypertension is present. There is marked thickening of the walls of the arterioles and of the small arteries of the polycystic kidney, with consequent reduction in the ratio between the thickness of the wall and the diameter of the lumina of the vessels of the kidney. The high incidence of retinal sclerosis in these cases indicates that the process probably is associated with a generalized vascular disturbance.

AUTHOR'S SUMMARY.

TUMOR OF THE BRAIN WITH DISTURBANCE IN TEMPERATURE REGULATION. ISRAEL STRAUSS and JOSEPH H. GLOBUS, Arch. Neurol. & Psychiat. **25**: 506, 1931.

Strauss and Globus report three cases which they believe support facts in favor of the existence of a heat-regulating center in the hypothalamus. The first case

presented a rather indefinite and inconstant clinical picture of mental symptoms (confusion and disorientation) with neurologic signs—changes in the reflexes and gait and urinary disturbances. The only sign that was fairly constant was an elevated temperature of 102 F. At necropsy, multiple tumors (spongioblastomas) of the corpus callosum and septum pellucidum were discovered with invasion of the anterior horn of the left lateral ventricle, of the walls of the third ventricle and practically its entire floor.

In the second case, that of a man, aged 62, there was also a mixture of mental and neurologic signs and symptoms with an unaccountable elevation of temperature (from 102 to 103 F.). Necropsy, which followed a decompression operation, showed multiple tumors. One tumor (a spongioblastoma) was situated in the region of the right optic thalamus and hypothalamus, which were invaded from the walls of the third ventricle and extended as far as the tuber cinereum below and as high as the taeniae thalami above. Another tumor was situated in the third temporal convolution of the left hemisphere.

In the third case, that of a woman, aged 57, in which the prominent symptoms were sleepiness and mental derangement associated with an elevation of temperature (103 F.) and a slow pulse, a diagnosis of tumor of the third ventricle was made, which was substantiated by necropsy (a glioma in the region of the optic chiasm).

An important general conclusion from the study of the three cases seems to be justified that a tumor of the periventricular zone of the third ventricle and in the tuber cinereum causes "a disturbance in the function of the heat-regulating mechanism."

GEORGE B. HASSIN.

THE BRAIN AND THE CEREBROSPINAL FLUID IN EXPERIMENTAL CEREBRAL EMBOLISM. WILLIAM CONE and S. E. BARRERA, Arch. Neurol. & Psychiat. **25**:523, 1931.

By injecting cut up black silk suture material, poppy seed and paraffin melting at 45 C. into the carotid the authors produced aseptic emboli in the brains of dogs. The animals were allowed to survive for variable periods up to seven days. Before they were killed, a cisternal puncture was performed. Twenty dogs that showed clinical signs of cerebral embolism were killed from eight and one-half to ninety-six hours after operation; one was killed after seven days. Small branches of the middle cerebral artery were usually blocked, causing areas of softening in the basal ganglions or the ependyma and the adjacent tissues. The spinal fluid cells were studied according to the method of Alzheimer. Polymorphonuclear neutrophilic leukocytes were present in the perivascular spaces of the infarcted areas and in the meninges (over the embolic area); the number of leukocytes increased during successive days, reaching its height at the end of forty-eight hours. After this time, they degenerated. They disappeared after seven days, granule cells replacing them in the softened area. A remarkable phenomenon was evident: The perivascular spaces of the cerebral blood vessels, filled with polymorphonuclear leukocytes, were seen plunging into the subarachnoid spaces. Organisms were not present in any of the animals. The astrocytes were badly damaged; their perivascular feet were broken up, thus disrupting the glial barrier and permitting the invasion of the cerebral parenchyma by mesodermal, polymorphonuclear elements.

In human material, polymorphonuclear cells were also numerous in the infarcted areas (within five and one-half days after the apoplexy), and in each instance they were present in the leptomeninges.

GEORGE B. HASSIN.

THE INFLUENCE OF TESTICLE EXTRACT ON THE INTRADERMAL SPREAD OF INJECTED FLUIDS AND PARTICLES. D. C. HOFFMAN and F. DURAN-REYNALS, J. Exper. Med. **53**:387, 1931.

The experiments in this paper show that testicle extract causes particles of india ink and of Prussian blue to spread much more extensively through the

intercellular spaces than similar suspensions made with Ringer's solution. Methylene blue (methylthionine chloride, U. S. P.) inoculated intravenously localizes more extensively in areas receiving injections of tissue extracts than in control areas receiving injections of tissue extracts without enhancing power. Kidney extracts have this property to a less degree, whereas spleen extracts and blood serum are devoid of it. The spreading power of extracts is destroyed by heating at 60 C. for thirty minutes, as is also the power to enhance infections. The precise mode of action of the Reynals factor is not known, but the results of the experiments here presented suggest that it may depend at least in part on the property whereby testicle extract increases the spread of injected material and alters the permeability of tissue cells. It is not inconceivable that changes in permeability facilitate the passage of vaccine virus through the endothelial cells of the blood and lymph vessels, and lead to the generalized vaccinia which is of frequent occurrence in the reported results. It has been shown that fluids and suspensions of inert particles are spread by the extract. *B. tetanus* and *B. coli* exotoxins and trypsin were not enhanced in their action despite the fact that they were spread through a more extensive area in the tissues. Viruses, on the other hand, are markedly influenced and in this respect resemble bacteria, not toxins and enzymes. It appears probable that a definite capacity for multiplication on the part of an injected substance is required if its pathogenic effects are to be enhanced. It may be concluded tentatively that the enhancing power of the testicle extract may depend on that property which not only spreads the injected material through a larger area but renders the tissue cells more easily penetrable by the agents.

AUTHORS' SUMMARY.

THE SKIN REACTIONS PRODUCED BY ALTERNATIONS OF HEAT AND X-RAY AT VARIOUS TIME INTERVALS. J. A. HAWKINS, J. Exper. Med. **53**:405, 1931.

Areas on the abdomen of the same guinea-pig were exposed to suberythemal doses of soft x-rays, to heat of an intensity below the critical dose for the production of burns and to both radiations in sequence with various time intervals between the exposures. The only effect of exposure to x-ray or heat alone was a slight scaling of the skin. The areas exposed to heat and roentgen radiation developed well marked and persistent burns when the exposure to one agent was made within three hours of the other. Scaling of the skin developed when the exposure to one agent was made one day after the other. This scaling was more marked and lasted longer than the scaling produced by either agent alone. The results were the same no matter in which sequence the agents were applied.

AUTHOR'S SUMMARY.

EFFECTS OF THE INTRAVENOUS INJECTION OF COLLOIDAL SILVER UPON THE HEMATOPOIETIC SYSTEM IN DOGS. S. S. SHOUSE and G. H. WHIPPLE, J. Exper. Med. **53**:413, 1931.

Colloidal silver has no specific action on the bone marrow of dogs but is a systemic poison which may cause anorexia, weakness, loss of weight, anemia and death. Hemolysis can be demonstrated after the administration of large doses of colloidal silver, and the anemia presumably is due, in part at least, to a destruction of red blood cells in the peripheral circulation. The colloidal silver, injected intravenously, is deposited as granules almost exclusively in the cells of the reticulo-endothelial system, after the manner of particulate substances. Repeated injections of nonlethal amounts of this substance are invariably followed by hyperplasia of the bone marrow. In no case was aplasia found. Large single doses of this material cause rapid death in twelve hours or less, characterized by pulmonary edema and congestion. An initial increase in the number of erythrocytes and leukocytes may occur following the use of smaller amounts of silver, but repeated injections cause considerable anemia, without a definite increase in the leukocytes and with no signs of a deficiency in blood platelets.

AUTHORS' SUMMARY.

APLASIA OF MARROW AND FATAL INTOXICATION IN DOGS PRODUCED BY ROENTGEN RADIATION OF ALL BONES. S. S. SHOUSE, S. L. WARREN and G. H. WHIPPLE, J. Exper. Med. **53**:421, 1931.

Constant results were obtained in the acute reaction to the specified amount of heavily filtered radiation over the bony skeleton. On the eighth or ninth day after exposure to the radiation, a short and fatal intoxication develops without warning. A profound leukopenia appears after from five to six days and is maintained in the peripheral blood (200 white blood cells or less per cubic millimeter) for the two or three days before death. The platelets suddenly disappear from the blood smears the day before death. This has some bearing on the life cycle of the platelet. All of the organs and structures of the body present extensive generalized capillary hemorrhage of recent origin. The substance of the spleen and lymph nodes is greatly reduced, and the germinal centers are visible only as remnants. The red cell hematocrit reading drops from about 50 per cent, or normal, to approximately 40 per cent. The bone marrow is depleted of all its cells except the connective tissue and fat cells, blood vessel endothelium, phagocytes filled with brown granules and occasional normoblasts.

AUTHORS' SUMMARY.

THE COMBINED EFFECTS OF COLLOIDAL SILVER AND HIGHLY FILTERED ROENTGEN RADIATION UPON THE HEMATOPOIETIC SYSTEM IN DOGS. S. S. SHOUSE and S. L. WARREN, J. Exper. Med. **53**:437, 1931.

The individual destructive effects of colloidal silver and heavily filtered radiation are still evident when the two are used together. The combined effects are cumulative in that small doses are more destructive than when either is used alone. The leukocytosis resulting from the injection of the colloidal silver affords no protection against the terminal leukopenia following the irradiation.

AUTHORS' SUMMARY.

THE TOXICITY OF CERTAIN NORMAL SERA FOR THE GUINEA PIG. M. E. FIELD, J. Immunol. **20**:89, 1931.

Lethal toxicity of human and rabbit serum persists for at least seventy-two hours. This toxicity decreases with age, so that serum one week old is usually completely innocuous. Primarily toxic normal serum causes a marked rise in the pulmonary arterial pressure, accompanied by bronchoconstriction. Nonagglutinating serum acts in every way as does normal serum. The cellular extract of hemolyzed guinea-pig cells is very toxic for the guinea-pig, causing a great rise in the pulmonary arterial pressure, followed by death. Toxic serum causes great intra vitam hemolysis which decreases in amount as the toxicity of the serum is attenuated by time.

AUTHOR'S SUMMARY.

FIBRINOPENIA. R. JÜRGENS and H. TRAUTWEIN, Deutsches Arch. f. klin. Med. **169**:28, 1930.

Jürgens and Trautwein review the three cases of hemorrhagic diathesis with complete or almost complete disappearance of the fibrinogen from the blood reported in the literature and to these add the clinical history of a fourth case, the first one ever observed in an adult. Examination of the blood revealed a great deficiency of fibrinogen but a normal amount of all the other components of coagulation. Postmortem examination disclosed an almost completely isolated, extensive carcinosis of the bone with fibrous transformation of the bone marrow. The primary growth was a carcinoma of the prostate. The macroscopic and particularly the microscopic changes indicated that the bone marrow plays a part in the formation of fibrinogen.

Pathologic Anatomy

Is Familial Jaundice of New-Born Infants Erythroblastosis? William L. Buhrman and Heyworth N. Sanford, Am. J. Dis. Child. **41**:225, 1931.

Two cases of fatal jaundice in new-born infants are reported. Both cases showed a familial history of jaundice at birth. The clinical picture was one of familial jaundice with deep progressive icterus, enlarged liver and spleen, bile pigments in the urine and an indirect van den Bergh reaction. Both cases were characterized by an intense anemia with extremely numerous nucleated red cells. In one case the time of bleeding was greatly increased and there was a reduction in the blood platelets. Both patients died, and autopsy showed erythropoiesis, blood-forming islands in the liver and hyperplasia of the bone marrow.

Authors' Summary.

The Involvement of the Coronary Arteries in Acute Rheumatic Fever. S. R. Slater, Am. J. M. Sc. **181**:203, 1931.

Three cases of rheumatic coronary arteritis with closure are reported. The symptomatology and the prognosis are discussed.

Author's Summary.

Pancreatitis Complicating Mumps. M. B. Brahdy and I. H. Scheffer, Am. J. M. Sc. **181**:255, 1931.

Pancreatitis complicating mumps produces a characteristic symptom-complex. It occurs in both sexes and at any age. In our series the extremes of age were 6 and 30 years. The onset is sudden between the fifth and eleventh days of the illness with nausea, anorexia and fever. The temperature rises in a day or two to 103 or 104 F. in the moderately severe cases, and returns to normal in from three to five days. The pulse is usually slow in relation to the temperature. The patients complain of malaise, nausea, headache and abdominal pain, and usually vomit. The pain and tenderness in our cases were always in the upper part of the abdomen. Most of our patients were constipated, and diarrhea, which has been reported by other observers, did not occur in our series. There was no glycosuria. Information from autopsy examinations is limited in two cases. The pathologic changes in the pancreas reported by Lemoine and Lapasset are comparable to the changes found by Wollstein in the parotid glands of monkeys experimentally inoculated with mumps. The abnormal findings in the pancreas described by the French authors are in all probability only a little more advanced than those which occur in the patients who recover.

Authors' Summary.

Diffuse Progressive Degeneration of the Gray Matter. Bernard J. Alpers, Arch. Neurol. & Psychiat. **25**:469, 1931.

In a carefully studied brain from a 4 months old infant which had had convulsions, restlessness, frozen pupils and practically no other physical neurologic findings, Alpers found a large number of pathologic conditions. They were present mainly in the cortex, basal ganglions and pons, and were classified as developmental and structural. In the former he included paucity of ganglion cells in the third cortical layer of the frontal, parietal and occipital lobes; accumulation of foci of embryonic cells (probably ependymal spongioblasts) underneath the ependyma, mostly of the frontal parts of the brain and one large focus of such cells in the parolfactory area; an immature state of the macroglia and ganglion cells some of which had a moth-eaten cytoplasm; demyelination of the cortex, corpus striatum and optic thalami with immense masses of blood vessels and capillaries many of which were budding. An interesting feature was an abundance of ganglion cells in the white matter of the brain and the cerebellum. The subependymal region was thickly covered with masses of spongioblasts, some of

which were rather remote from the ependyma. In addition there were evidences of arteriosclerosis in a pial blood vessel.

The structural changes were as foci of softening or necrosis, mostly of atypical nature, incomplete and confined to the cortex and the basal ganglions. The optic thalamus contained a typical focus with gitter cells and a marked mesodermal reaction which was absent in the atypical foci. In general, the reaction to the vast parenchymatous lesion of the brain was neuroglial, not mesodermal. The corpus striatum and the walls of the blood vessels contained lipoids. Alpers sums up his observations as an incomplete necrosis of the brain described by Jakobs. He quotes two more similar cases studied in the latter's laboratory and contrasts them with the diffuse periaxile sclerosis of Schilder where the white matter is principally affected, while in the three cases from Jakob's laboratory the process affected mainly the gray matter. The problematic cause of the cortical destruction is likely an intoxication.

GEORGE B. HASSIN.

DISSEMINATED ENCEPHALOMYELITIS. ROY R. GRINKER and PETER BASSOE, Arch. Neurol. & Psychiat. **25**:723, 1931.

The authors report four cases of a diffuse involvement of the cerebrospinal nerve with the main view to a broad pathologic classification of various types of encephalitis. In the first patient, five days after a respiratory infection and bulbar symptoms and symptoms of the spinal cord developed. Death occurred from pneumonia. The pia-arachnoid showed lymphocytes mixed with granule cells, the blood vessels were engorged; the parenchyma of the brain and spinal cord, and especially the white matter of the medulla and the sacral portion of the spinal cord, was covered with foci of softening situated around the blood vessels (mostly veins); lymphocytes and plasma cells were also seen around the blood vessels, but fat-filled cells were much more numerous. The authors consider this case not one of disseminated encephalomyelitis, but rather of the same type as cowpox encephalitis.

In case 2, a gradual hemiplegia developed in a child, aged 10. Death occurred twenty-three days after a progressive course and a temperature of 103 F. Microscopic examination revealed areas of demyelinization in the subcortex, with many myelin fibers preserved and without relationship to the blood vessels. The latter showed granule cells (in the damaged areas, while in remote regions they exhibited hematogenous elements mainly). While the infectious character of the lesions is not denied by the authors, the toxic degenerative nature of the foci of softening is not readily admitted by them.

In case 3, in which the authors made the diagnosis of subacute disseminated encephalomyelitis, the duration was five months, after a sudden onset with vertigo, vomiting and diplopia. Three weeks later the acoustic, facial and right sixth nerves became involved, followed by ataxia in the right leg and arm and hemiparesis of the left side of the body. About one month before death, clonic spasms in the right arm appeared, followed by paralysis. The general condition grew much worse; difficulties of speech set in, with optic neuritis and bulbar phenomena followed by death. Necropsy revealed in the region of the pons, medulla and cerebral peduncles patches of demyelinization. The affected areas contained fat scattered in the tissues and in the perivascular spaces of the adjacent blood vessels mixed with lymphocytes, but the patches had "no correlation with a definite arterial supply." Older patches contained fibroblasts and small amounts of reticulin.

In the fourth case, a staggering gait and bulbar symptoms with inability to control the right arm in writing developed in the patient two months after a febrile attack. The symptoms gradually increased within the next two years. Examination revealed diplopia, nasal speech, paralysis of the left vocal cord and left facial nerve (peripheral type) with a right hemiparesis. While the patient was in the hospital a febrile attack developed with aggravation of the general condition; the patient left unimproved. On the basis of three cases examined

pathologically a classification of various types of encephalomyelitis has been attempted. The greatest majority, including such disease processes as multiple sclerosis and Schilder's disease, cannot, according to the authors, be considered primarily degenerative, and the presence of hematogenous elements in the perivascular spaces should not be overestimated in diagnosing a case as due to an inflammatory process.

GEORGE B. HASSIN.

ENCEPHALITIS AND ENCEPHALOMYELITIS IN MEASLES. ARMANDO FERRARO and I. H. SCHEFFER, Arch. Neurol. & Psychiat. **25**:748, 1931.

Six cases of lesions of the central nervous system following measles have been studied. Though somewhat varying in severity, the changes were practically alike. They were: perivascular demyelinization, though foci with loss of myelin were present away from the blood vessels, perivascular proliferation of microglia, mild and only occasionally hematogenous (lymphocytes, occasionally plasma cells) perivascular infiltrations, mild meningeal changes and parenchymatous changes varying from acute swelling of ganglion cells to their liquefaction. The vessels were engorged, sometimes thrombosed and proliferated. The macroglia showed both proliferative and regressive changes. The perivascular proliferation of the microglia was mainly in the white substance. The type of changes is classified as encephalopathy instead of encephalitis, because of the practical absence of hematogenous elements in the perivascular spaces. Whether the cause is a direct action of the virus of measles, a toxin or some allergic phenomenon is left unanswered.

GEORGE B. HASSIN.

CEREBELLAR HEMANGIOBLASTOMAS WITH INCIDENTAL CHANGES OF THE SPINAL CORD. C. DAVISON, W. SCHICK and S. P. GOODHART, Arch. Neurol. & Psychiat. **25**:783, 1931.

In the first patient, a woman, aged 42, diplopia developed thirteen years before admission to the hospital, accompanied by dizziness and tinnitis; one year later vision became impaired, while the diplopia was intermittent. About three years before admission, the patient had difficulties in walking and urinary disturbances and vomited. Examination at this time revealed right optic atrophy, absent vibratory sense, a right supranuclear facial paralysis, horizontal nystagmus and signs of a bilateral lesion of the pyramidal tract. The patient entered the hospital with the complaint of difficulty in walking, diplopia and visual disturbances. In addition to the foregoing signs and symptoms, examination revealed a left papilledema and a poor pupillary reaction to light. The spinal fluid and blood gave a negative Wassermann reaction. Pleocytosis in the spinal fluid with a large number of polymorphonuclears, but no albumin, and large quantities of sugar in the urine were the other noteworthy features. The condition grew worse; paraplegia in flexion developed, but the spinal fluid became entirely normal. A multiple lesion of the cerebrospinal nervous system was diagnosed. Necropsy revealed hydrocephalus, secondary to an encapsulated cyst in the left lobe of the cerebellum which contained a vascular mural tumor (hemangioblastoma) the size of a hazelnut and which obstructed the sylvian aqueduct and the fourth ventricle, and multiple sclerosis.

In the second case that of a man, aged 44, there was vertigo, gastric distress, unsteady gait, diplopia and genito-urinary anomalies. Later bulbar symptoms and an exophthalmos developed, and the patient was unable to walk or to sit up. There was muscular atrophy with fibrillation in the pectorals, calf and abductor muscles of the thigh; exaggerated tendon and diminished skin reflexes, intention tremor, sensory disturbances in the right lower extremity, a mild bilateral papilledema, horizontal nystagmus with a right supranuclear facial paralysis. Necropsy showed a right cerebellopontile tumor (hemangioblastoma) partially adherent to the eighth nerve and to the fibers of the ninth and tenth nerves; the spinal cord showed foci of necrosis in the middorsal segment.

GEORGE B. HASSIN.

PATHOLOGY OF SCLERODERMA. G. RAKE, Bull. Johns Hopkins Hosp. **48**:212, 1931.

A case of scleroderma associated with Raynaud's disease and dilatation of the esophagus and colon is described in a woman, aged 30 years. Lesions were present in the cervical sympathetic ganglion cells, and the peripheral arteries showed an endarteritis. The relation of the anatomic findings to the clinical symptoms and signs is discussed, and the present state of our knowledge on the subject is reviewed.

AUTHOR'S SUMMARY.

INCREASE IN HEART VOLUME IN MITRAL STENOSIS. E. F. BLAND, G. M. BALBONI and P. D. WHITE, J. A. M. A. **96**:840, 1931.

A young man with mitral disease, under observation for nineteen years, lived a relatively active life up to the time of his sudden death, in spite of an extraordinary amount of cardiac enlargement and an extreme degree of auricular dilatation.

The volume (displacement) of the filled heart was 4,600 cc., which is from six to seven times that of a normal sized heart and establishes a record for future comparison. The literature is notably deficient in measurements of heart volume (displacement), a determination that should be a helpful indication of the degree of responsibility of cardiac dilatation in the production of cardiac enlargement. In the case recorded, cardiac hypertrophy was far less a factor than dilatation in causing the extreme enlargement; the weight was 850 Gm., which has often been exceeded.

The left auricle had a capacity of 1,760 cc., a measurement exceeded by only three other cases in the literature. The right auricle held 650 cc., which appears to be a record capacity.

AUTHORS' SUMMARY.

THE NUMBER OF OPEN GLOMERULI IN ACUTE MERCURIC CHLORIDE NEPHROSIS. R. A. MOORE and L. M. HELLMAN, J. Exper. Med. **53**:303, 1931.

Acute mercurial nephrosis in the rabbit is not associated with a decrease of glomerular circulation.

AUTHORS' SUMMARY.

THE STRUCTURE OF YOUNG BLOOD PLATELET THROMBI. K. APITZ, Centralbl. f. allg. Path. u. path. Anat. **50**:9, 1930.

Alcohol from 75 to 98 per cent, was painted on the abdominal veins of rabbits, allowed to act from thirty to sixty seconds and then the veins were excised at intervals of from five to one hundred and twenty minutes. In the early stages of thrombi, platelets were deposited on the wall of the injured vein in string-of-pearl fashion with red blood cells occupying the meshes. Later a contraction took place so the red cells were squeezed out and a highly nuclear platelet mass remained. In serial sections, fibrin was never found between the platelets in the mass, although in three of twenty-one veins fibrin threads were found between the red cells at the periphery of the mass. In another series of experiments injections of novirudin were made into the animals in addition to injuring the veins with alcohol. In the second series no typical collections of platelets occurred at the site of injury, although no changes were noted in the platelets themselves. The conclusion is reached that the plasma ordinarily contributes to the agglutination process of platelets in some way, and this property is temporarily checked by substances which prevent clotting.

GEORGE RUKSTINAT.

IDIOPATHIC CALCIFICATION OF THE CEREBRAL VESSELS. T. FAHR, Centralbl. f. allg. Path. u. path. Anat. **50**:129, 1930.

Disseminated calcification was observed in the capillaries and precapillaries in the white matter of the brain of a man 55 years old. Grossly, this was

evident as tiny thornlike processes about which there was no degeneration. Microscopically, necrosis was not observed but occasional small hemorrhages were seen. The only other disease found at autopsy was a chronic cholelithiasis and a hypostatic bronchopneumonia. In attempting to explain the condition, Fahr hints that the deceased may have imbibed vigantol-like substances which predisposed to calcification because he was known to partake of a variety of medicines frequently.

GEORGE RUKSTINAT.

PANMYELOPHTHISIS (ATROPHY OF BONE MARROW). B. KARITZKY, Centralbl. f. allg. Path. u. path. Anat. **50**:177, 1930.

The patient in whom this illness was studied was a woman 20 years old in whom severe epistaxis, metrorrhagia and melena developed. When first seen her blood picture showed 49 per cent hemoglobin, 2,100,000 red blood cells per cubic millimeter, 2,100 white blood cells per cubic millimeter, 89 per cent lymphocytes and 9 per cent polymorphonuclears. Despite a liver diet, roentgen therapy and three transfusions of blood, her condition became rapidly worse; a month and a half after her entrance to the clinic, the red blood cells were only 505,000, the white blood cells, 1,400, the polymorphonuclears, 36 per cent and the lymphocytes, 61 per cent; death from anemia occurred. At autopsy, hemorrhages were noted in the bowel, especially at the tips of the rugae and about the lymph follicles and in the bone marrow of the femurs. There was a marked hemosiderin pigmentation of the cardiac muscle, spleen, kidney and liver and localized lymphocytic infiltrations in the liver. The author considered the possibility of the transfusions of blood leading to the deposits of hemosiderin, but eventually concluded that they were best explained by the anemia. Although the case reported does not fit in, in entirety, with those reported by Frank, the author thinks that it belongs with "panmyelophthisis" rather than with pernicious anemia.

GEORGE RUKSTINAT.

ABNORMAL ORIGIN OF THE LEFT CORONARY ARTERY FROM THE PULMONARY ARTERY. A. J. SCHOLTE, Centralbl. f. allg. Path. u. path. Anat. **50**:185, 1930.

In the body of a 2½ month old girl who died of bronchopneumonia, the left coronary artery originated from the sinus of the left pulmonic leaflet. Associated with it were: a marked fibrous myocarditis of the left ventricle with fresh regions of focal necrosis; fatty changes and calcification of the cardiac muscle, and diffuse endocardial thickening of the left ventricle.

GEORGE RUKSTINAT.

THE PATHOGENESIS OF PERFORATING PURULENT AORTITIS AND ITS SEQUELAE. J. F. BUCHALY, Centralbl. f. allg. Path. u. path. Anat. **50**:225, 1930.

Acute nonspecific aortitis can be divided on the basis of pathologico-anatomic changes into verrucose, ulcerating or phlegmonous, and etiologically into the metastatic-embolic types and those occasioned by proximity to an infective focus. The author contributes the following interesting case report. A girl, aged 20, affected with chronic furunculosis, fell from a bicycle and injured her back. The injury was sufficient to produce a locus minoris resistentiae, and a staphylococcic abscess formed behind the abdominal aorta. From this abscess a phlegmonous panaortitis developed, ulceration occurred, and a small amount of bleeding took place into the abscess cavity forming a spurious aneurysm. Either at this time or previous to it a septicopyemia had occurred. Then rupture of the aorta occurred into the false aneurysmal sac extended above the diaphragm, and death resulted from bleeding into the right pleural cavity.

GEORGE RUKSTINAT.

THE PATHOLOGIC ANATOMY OF PARAFFIN SICKNESS. N. M. KOLESNIKOW, Centralbl. f. allg. Path. u. path. Anat. **50**:277, 1931.

The investigations on this illness were carried out on workers in the paraffin factory at Graseny. Clinically, the illness manifests itself as miliary comedones, acne, pustules and papules on the skin. The sites of predilection are the shoulders, upper part of the back, thighs and neck — those parts subject to rubbing. The first illness usually starts from two to eight weeks after beginning the work, but in some persons a rash developed as much as four months after stopping work. In biopsy material it was found that the disease starts as an infection of the hair follicles. The lumen of the follicle becomes widened due to the pressing into it of paraffin products. The hair roots become markedly altered, stain more intensely with basic dyes, become brittle and break into small scales. The walls of the distended follicles become thin and then they loose their connection with the epidermis and lie in the cutis in the form of large thin-walled sacs. The surrounding tissue becomes infiltrated with lymphocytes, histiocytes and foreign body giant cells. In some cases the follicles produce a leukocytic reaction, which in a short time leads to their destruction. Clinically, this is manifested in pustules. The follicles or their remnants after acting as foreign bodies in the tissues may lie a long time without causing any reaction. Due to many agencies, inflammation may set in at any time subsequently. In this way new or delayed eruptions are explained. The remnants of follicles eventually become surrounded by firm connective tissue into which they are absorbed. The end-result of the process is diffuse scar formation similar to scleroderma.

GEORGE RUKSTINAT.

THE EFFECT OF LIVER TREATMENT ON THE ANATOMIC CHANGES IN PERNICIOUS ANEMIA. T. FAHR, Deutsche med. Wchnschr. **57**:8, 1931.

Fahr has found that under the influence of liver treatment in pernicious anemia, the characteristic anatomic changes in the marrow, the heart and the liver disappear. The liver treatment, however, does not seem to have any decisive effect on the changes in the spinal cord that may arise in the course of the disease.

Pathologic Chemistry and Physics

THE ACTION OF ANIMAL TISSUES ON THE SILICIOUS SPICULES OF A FRESH-WATER SPONGE. RALPH G. MILLS, Am. J. Hyg. **13**:224, 1931.

The spicules of the fresh water sponge, *Spongilla fragilis,* are composed of opal, a form of hydrated silica, which is similar to quartz in its chemical reactions. These spicules, when introduced into the tissues of animals, are slowly but definitely dissolved, proving that silica is soluble in the tissue fluids of animals and presumably of man. This confirms the assumption held by many students of silicosis that particles of silicious material are gradually converted into the colloidal state and, as such, exert a fibroplastic influence on the lungs of miners and other industrial workers exposed to silicious material. Definite fibrosis of the lung of a dog into which the spicules had been introduced suggests that there is a concomitant injury attributable to the disappearance by solution of the silicious elements of the spicules.

AUTHOR'S SUMMARY.

THE VALUE OF BRAIN LIPOIDS AS AN INDEX OF BRAIN DEVELOPMENT. FREDERICK TILNEY and JOSHUA ROSETT, Bull. Neurol. Inst. New York **1**:28, 1931.

A brain has been judged efficient or deficient by different investigators according to its size, weight or anatomic or chemical properties. In the present study, Tilney and Rosett give their preliminary observations of the lipoid contents of the brain which are so important in the development of myelin and with it in that

of the brain. By using Baug's micromethod for the determination of the lipoids in the blood they found that different divisions of the cerebral cortex possess different chemical constituents. Their study is based on sixty-eight human brains fixed in formaldehyde. They admit the shortcomings of this method, for fixation in formaldehyde affects the phosphatides, a varying amount of phosphorus being lost from the tissues. Yet they arrived at certain highly instructive conclusions. They found that after two years the values of the lipoids remained fairly constant. In ten cases studied for the relative lipoid and water contents in the two halves of the cerebral and cerebellar hemispheres, the average difference was less than 0.1 per cent. Profound systemic changes, as well as defective nutritional states, deficiency of vitamins and narcotics such as ether diminish the lipoids in the brain. These are also slightly diminished in infections (pneumonia, tuberculosis, measles, etc.) and very much diminished in nutritional and metabolic disorders. In diseases of the blood and of the cardiovascular organs and in neoplastic diseases the lipoid contents are somewhat increased, with a corresponding decrease in the water content. In lesions of the nerves with pronounced parenchymatous degeneration, the lipoid content is, with few exceptions, practically unchanged. In intrinsic diseases of the cerebrum and in general systemic diseases, the content of alcohol-soluble fatty substances in the brain is increased, indicating a pronounced degenerative condition — the presence in the brain of an abnormal fatty compound. Further studies are to deal with "fractional and topographical estimations of brain lipoids in embryonic, fetal and postnatal stages of development." A special contribution will deal with the lipoid content of the blood, spinal fluid and central nervous system in patients with multiple sclerosis.

GEORGE B. HASSIN.

THE PLASMA PHOSPHATASE IN DISEASE, PARTICULARLY IN BONE DISEASE. H. D. KAY, J. Biol. Chem. **89**:249, 1930.

The phosphatase activity of the blood plasma, expressed by its ability to hydrolyse betaglycerophosphate, becomes increased in generalized diseases of the bones, such as osteitis deformans, generalized osteitis fibrosa, osteomalacia and rickets. The values may be increased, in extreme conditions, to more than twenty times those characteristic of normal plasma. The increase is correlated, in a general way, with the severity of the disease.

E. R. MAIN.

THE CALCIUM CONTENT OF STRIATED MUSCLE OF RACHITIC ANIMALS. V. G. HAURY, J. Biol. Chem. **89**:467, 1930.

The calcium content of the striated muscles of the rachitic rat is 44 per cent lower than that characteristic of the muscles of the normal rat.

E. R. MAIN.

SUGAR CONTENT OF SKIN AND MUSCLE IN DIABETIC AND NON-DIABETIC PERSONS. H. C. TRIMBLE and B. W. CAREY, JR., J. Biol. Chem. **90**:655, 1931.

The elevation in the concentration of sugar in the blood which characterizes diabetes is accompanied by a marked increase in the sugar contents of the skin and muscle. The average sugar contents of the skin and muscle of nondiabetic persons are 56 and 28 mg. per hundred cubic centimeters, respectively. In diabetes, these averages may reach 144 and 51 mg.

ARTHUR LOCKE.

THE OXIDATION OF FATTY ACIDS OF THE SERUM IN ANEMIA. G. P. WRIGHT and B. ARTHUR, J. Biol. Chem. **90**:757, 1931.

The blood serum of rabbits rendered anemic through direct loss of blood has an increased content of unsaturated fatty acids owing to the extension of erythropoietic tissue and the consequent extrusion of marrow fat into the blood.

In the presence of ferricyanide, these unsaturated fatty acids consume oxygen and their presence may lead to errors when the determination of the oxygen content of such blood is attempted by the usual Haldane procedure.

ARTHUR LOCKE.

PRECIPITATION WITH CEREBROSPINAL FLUIDS. A. M. MALLOY, R. L. KAHN and LUCY WESTALL, J. Infect. Dis. **48**:203, 1931.

The Kahn reaction and the colloidal gold and mastic reactions depend on the globulin fraction of the spinal fluid, the albumin fraction giving negative results. The globulin fraction gives more sensitive results than the unfractionated fluid, owing undoubtedly to the removal of the protective action of the albumin. After a Kahn test has been performed with a solution of globulin obtained from spinal fluid and the precipitate removed by centrifugation, the supernatant fluid continues to give colloidal gold and mastic reactions. This indicates that the substance or condition responsible for the Kahn reaction is not the same as that responsible for the colloidal gold and mastic reactions. Heating the globulin fraction at 56 C. for as long as two hours exerts little effect on the three reactions. Heating for twenty-four hours decreases the sensitivity, especially of the Kahn reaction. Heating at 65 C. for thirty minutes reduces the sensitiveness of the Kahn reaction more than that of the other two. Heating at 70 C. for thirty minutes reduces the sensitivity of all three reactions. The experiments with heating also indicate that the conditions governing the Kahn reaction are not identical with those governing the colloidal gold and mastic reactions.

AUTHORS' SUMMARY.

THE BLOOD-PIGMENT IN OBSTRUCTIVE JAUNDICE. W. J. GRIFFITHS and GEOFFREY KAYE, Brit. J. Exper. Path. **11**:441, 1930.

In six rabbits, ligation of the common bile duct was followed by early and definite icterus, the serum giving a prompt direct van den Bergh reaction. Directly reacting human serum and the serum of rabbits after ligation of the common bile duct resemble in their properties a solution in serum of the "direct reaction" pigment that we have shown to be present in bile. Indirectly reacting human serum resembles in its properties a suspension in serum of pure bilirubin. We have shown that the "direct reaction" pigment yields a diazoreaction over a wide range of hydrogen ion concentrations, viz., when the hydrogen ion concentration of the mixture after addition of the reagent lies between p_H 1.2 and p_H 5, approximately; on the other hand, the pigment of indirectly reacting serum, like bilirubin, yields no prompt direct reaction until the final hydrogen ion concentration of the mixture is adjusted to about p_H 6 by the addition of alkali to the serum before it is mixed with diazoreagent.

AUTHORS' SUMMARY.

THE SUGAR CONTENT OF PLEURAL AND PERITONEAL EXUDATES. J. HERMS, Beitr. z. Klin. d. Tuberk. **75**:748, 1930.

An increase in the total number of cells and in the number of polymorphonuclears in pleural and peritoneal exudates indicates intensified inflammatory processes. The protein content is not a reliable criterion, because an increase may be due either to inflammation or to resorption. The main portion of reducing substances is dextrose; its amount in exudates is independent of the amount of dextrose in the blood; it may be equal to that in the blood or may be reduced to 10 mg. The decrease of dextrose in exudates is caused by fermentative anaerobic glycolysis, by adsorption on proteins and by increase of the amount of fluid. Low dextrose values are found in mixed infections, in exudates in chronic pneumothorax, in exudates that become rapidly purulent and in empyemas. Peritoneal exudates rarely show low dextrose contents.

MAX PINNER.

HEAVY METALS IN GALLSTONES. R. SCHÖNHEIMER and W. HERKEL, Klin. Wchnschr. **10**:345, 1931.

Copper, zinc, manganese and iron were demonstrated in appreciable amounts in gallstones. In comparison with the concentrations of copper and zinc in the liver, that of copper in the gallstones is greater, and that of zinc less.

EDWIN F. HIRSCH.

Microbiology and Parasitology

THE FILTRABLE FORMS OF BACTERIA. P. HADLEY, E. DELVES and J. KLIMEK, J. Infect. Dis. **48**:1, 1931.

In this investigation we have, for the first time we believe, generated artificially, and subsequently cultivated in pure lines, the filtrable, virus-like stage of a bacterial species. We produced this form (G type culture) by forcing the dissociative reaction on the mother S or R type culture. We grew this G form in pure cultures as a distinct cyclostage before demonstrating that at least some of the cellular elements contained in it were invariably filtrable through Berkefeld N and W candles, as opposed to the S and R type cells, which are not filtrable under the same conditions. From the filtrates we were successful in recovering the G type culture. We have demonstrated, moreover, that the visible elements of the filtrable phase are very different culturally, morphologically, biochemically, serologically, immunologically and in their relation to the Shiga bacteriophage from the "normal" forms of the Shiga species, but that they may be caused to "revert" to the original cell type, possessing all of the original characteristics, including toxicity and susceptibility to bacteriophagic influence. We have shown that the filtrable bodies experience a phase of existence in which they are not visibly cultivable in or on the usual culture mediums and by the usual methods, and that a special technic, such as that used by Hauduroy, must be employed for bringing them into visible development. We have demonstrated, in addition, the high degree of stability enjoyed, in many cases, both by the G type cultures and by the associated virus-like forms when sealed in ampules and stored for periods exceeding two years; we have come to regard them as the most stable forms of the Shiga species. Besides demonstrating the filtrable stage for the Shiga bacillus, we or other members of our laboratory group have shown the existence of analogous phases of culture development in the ontogeny of ten other bacterial species. Their G forms are equally filtrable. Regarding the nature of the G type cultures and the associated filtrable forms, we have concluded that they represent definite cyclostages in the ontogeny of the Shiga species, and that they constitute, in part, at least, the gonidia and the microgonidia. We regard the G type cultures as the visible stage of culture existence lying between the noncultivable (visibly) virus form and the ordinary culture type (S). Both, therefore, have a definite relation to microbic dissociation and are natural products of the dissociative mechanism. The exact cytologic origin of the G forms and the associated virus bodies is not yet entirely clear. There may be several modes of origin, and these may not be the same in different bacterial species. The only origins of which we can speak with any degree of assurance are the lateral or terminal "buds" formed on the rods of old S cultures and the granular inclusions ("gemmules," "gonidia") produced in, and liberated by, the long rods and the filamentous structures that characterize the R type culture. Among the species that we have studied we regard this fungoid R phase as the reproductively mature culture form. The S, we believe, represents a lower, vegetative stage in the ontogeny of the species, while the G type, with its associated virus-like bodies ("microgonidia"), represents the lowest. The specific function of the S type cells is, therefore, vegetative growth; that of the R type cells, reproduction; that of the G forms, dissemination of the species. Regarding the significance of these new filtrable forms for bacteriology, epidemiology and pathology, although much must be left for future study, we have felt justified in discussing in a tentative manner some of the pos-

sibilities that have occurred to us. At the same time we observe that the filtrable phase of ontogeny is not limited to the intestinal bacteria; and it is possible that the analogous forms of other species may possess a different significance from that suggested by the Shiga bacillus, particularly from the point of view of virulence. Regarding the possible relation of these virus-like bodies to the large group of filtrable viruses, we believe that our results offer no grounds for conclusions. At the same time, they perhaps suggest that it will be of advantage, in the future, to incorporate the point of view of microbic dissociation in the study of so-called virus diseases.

AUTHORS' SUMMARY.

FILARIAL PERIODICITY. F. W. O'CONNOR, Porto Rico J. Pub. Health & Trop. Med. **6**:263, 1931.

Histologic studies suggest that the hypothesis of Clayton Lane to the effect that the phenomenon of filarial periodicity is due to a simultaneous cyclical parturition of gravid female filariae is correct. A mechanism by which the microfilariae migrate from the vicinity of the parent worms in the lumina of the lymphatic vessels through the walls of the same lymphatic vessels to the adjacent small blood vessels on their way to the general circulation has been demonstrated. While it is not claimed that this is the usual method of migration, the determined nature of its performance suggests that this might be the case.

AUTHOR'S SUMMARY.

Immunology

PROTECTION OF THE MONKEY BY ANTIPOLIOMYELITIC SERUM. C. P. RHOADS, J. Exper. Med. **53**:115, 123 and 137, 1931.

Experiments are reported on the use of poliomyelitis virus neutralized by specific antiserum as an agent to induce active immunity against the experimental disease in monkeys. The results indicate that protection can be conferred on a certain number of the treated animals. The neutralized material did not give rise to symptoms of the disease in any of the monkeys treated. Active poliomyelitis virus, suitably neutralized by admixture with convalescent serum, was without pathogenic effect when given by repeated nasal instillations.

The experiments described in this paper raise the question whether, therapeutically considered, the antipoliomyelitic horse serum should be regarded as an exact equivalent of, and hence employed as a perfect substitute for, convalescent serum. This question can only be answered by further experiment and observation.

A comparison has been made of the neutralizing value of pooled convalescent monkey serum for the filtered virus of poliomyelitis before and after a series of reinforcement injections of the same virus strain. The strength of the pooled convalescent serum is increased by the reinforcing procedure. The original monkey convalescent serum had a neutralization value much below that of a pooled human convalescent serum. By reinforcement, the neutralization value of the monkey serum was brought approximately to that of the human serum. One sample of serum from a supposedly normal child of 8 years exhibited a neutralizing value approximately equal to that of a pooled human convalescent serum and the reinforced pooled monkey serum.

AUTHOR'S SUMMARIES.

MOUSE PROTECTION TESTS FOR ANTIBODY IN PNEUMOCOCCUS PNEUMONIA. F. T. LORD and E. L. PERSON, J. Exper. Med. **53**:151, 1931.

Though in general in pneumococcus pneumonia the appearance of protective substance coincides rather sharply with the fall in the temperature, antibodies may appear spontaneously in the blood serum as early as the third or fourth day, and crisis and recovery may be delayed until between the sixth and the tenth day.

Recovery at times occurs without demonstrable protective substance in the blood of patients in whom protection later develops. The amount of antibody developed in the course of pneumococcus pneumonia is small, and in the majority of the patients tested was insufficient to protect against more than 100 lethal doses of homologous pneumococci and never against more than 10,000 lethal doses. Treatment with Felton's antibody late in the course of the disease materially increases the amount of protective substances in the blood. In fatal cases a high degree of protection may be established by treatment. After the third day, doses of more than 200,000 Felton units are usually necessary to produce a greater degree of protection than might otherwise be expected. The formation of protective substances by the patient himself is not an assurance against the progress of the infection to a fatal termination. Protective substance in the blood and pneumococcic septicemia may occur simultaneously.

AUTHORS' SUMMARY.

IMMUNIZATION AGAINST VACCINIA BY NON-INFECTIVE MIXTURES OF VIRUS AND IMMUNE SERUM. C. P. RHOADS, J. Exper. Med. **53**:185, 1931.

Vaccine virus and specific immune serum mixed in a proportion that will not produce a lesion when inoculated intradermally will induce immunity when instilled into the nasal cavities of rabbits. The mixture is also effective when inoculated subcutaneously. Immunity is brought about with a minimum systemic and no local reaction. When the mixtures are incompletely neutralized, generalized vaccinia results.

AUTHOR'S SUMMARY.

THE PULMONARY PERMEABILITY IN DOGS IN RELATION TO ANAPHYLACTIC SHOCK. N. P. SHERWOOD and O. O. STOLAND, J. Immunol. **20**:101, 1931.

Our results indicate that in heart-lung perfusion experiments on normal and sensitized dogs, the phenomena consisting of a reduction in rate of flow of perfusion fluid of from 50 to 75 per cent, the development of a rubbery consistency of the lungs and the appearance of a tracheal exudate, all in from four to seven minutes, are not peculiar to the sensitized dog as judged by the blood pressure and coagulation time phenomena. While these do not correlate with other and at present accepted criteria of sensitization, yet a quantitative study of our data suggests that a probable change in permeability in heart-lung tissue, perhaps independent of anaphylaxis, does frequently result from the injection of horse serum in a dog. Experiments in passive sensitization show that marked variation exists in either ability to take up antibody or refractoriness to the shocking dose of antigen. This observation indicates that negative results on a single recipient are not an accurate criterion of deficiency or absence of antibodies in the donor's blood.

AUTHORS' SUMMARY.

ISOHEMAGGLUTINATION: THE WORK OF JAN JANSKÝ WITH A CRITICAL ANALYSIS. J. A. KENNEDY, J. Immunol. **20**:117, 1931.

This article reviews the original publication by Janský on isohemagglutination in *Archives Bohèmes de médécine clinique* (**8**:85, 1907).

THE ANTIGENIC PROPERTIES OF BACTERIOPHAGE. E. W. SCHULTZ, J. S. QUIGLEY and E. L. WOOLSEY, J. Immunol. **20**:149, 1931.

Judged by plaque counts, the neutralization of phage by antiphagic serum was found to follow quite closely the curve of the velocity of an adsorption reaction. No evidence was obtained to indicate that the reaction follows a logarithmic curve. Results were obtained which indicate that in the neutralization the reaction under set conditions takes place in definite proportions, provided the reaction is allowed to go to completion. The Danysz-Bordet effect was elicited, when phage was

added in small increments to serum and, similarly, when serum was added to phage in small increments. Neutral serum-phage mixtures could not be dissociated by dilution with physiologic solution of sodium chloride.

FROM AUTHORS' SUMMARY.

SKIN REACTIONS TO POLLEN EXTRACTS IN RABBITS. H. W. CROMWELL and M. B. MOORE, J. Immunol. **20**:161, 1931.

Skin sensitivity to pollen extracts has been demonstrated in actively immunized rabbits, trypan blue being used as an aid, after the method of Ramsdell (*J. Immunol.* **15**:305, 1928, and **16**:509, 1929). The reaction is specific, and is more delicate than the precipitin test as indication of an immune response of the animal. The localization of the trypan blue also occurs in the abdominal fluid of the immune animal following intraperitoneal injection of the pollen antigen. Passive transfer of the skin sensitivity from immune to normal rabbit has been accomplished by the use of large quantities of immune serum.

AUTHORS' SUMMARY.

SKIN REACTION TO AGAR AFTER INJECTION OF MENINGOCOCCUS FILTRATE. G. M. SICKLES, J. Immunol. **20**:169, 1931.

Intravenous injections of agar may be followed by reactions in skin areas prepared by injections of meningococcus toxic filtrate. Local reactions did not occur at the site of intracutaneous injections of agar when followed by intravenous injections of agar or of toxin. No reactions were obtained after the injection of other nonbacterial substances such as galactose, gelatin, serum and india ink, intravenously, following intracutaneous injection of meningococcus toxic filtrate.

AUTHOR'S SUMMARY.

PROMOTION OF PHAGOCYTOSIS BY VARIOUS CHEMICAL SUBSTANCES. RUTH TUNNICLIFF, J. Infect. Dis. **48**:161, 1931.

Rabbits receiving intravenous injections of solutions of calcium gluconate, calcium chloride, sodium salicylate, sodium iodide, dextrose and neoarsphenamine showed a marked increase in phagocytosis by leukocytes in the blood. Physiologic solution of sodium chloride, distilled water, milk, sodium phosphate and mercurochrome-220 soluble in water or in dextrose injected intravenously into rabbits appeared to cause no increase in phagocytosis. Intravenous and intramuscular injections of calcium gluconate produced the same high degree of phagocytosis in rabbits, intravenous injections acting twenty-four hours earlier than intramuscular injections. A high degree of phagocytic activity was obtained in a man given intravenous injections of calcium gluconate, followed by intramuscular injections and finally by the use of the drug by mouth. With the discontinuation of the drug, phagocytosis returned to normal. In vitro, dilute solutions of calcium gluconate, sodium iodide, dextrose and mercurochrome in dextrose stimulated phagocytosis, while solutions of sodium phosphate, milk and mercurochrome in water did not appear to increase phagocytic activity.

AUTHOR'S SUMMARY.

CUTANEOUS HYPERSENSITIVENESS IN GUINEA-PIGS INFECTED WITH BRUCELLA ABORTUS. A. C. S. STROEM, J. Infect. Dis. **48**:167, 1931.

Guinea-pigs infected with *Brucella* showed cutaneous hypersensitiveness to abortion even when gross anatomic lesions were absent; those infected with one strain of *Brucella* showed cutaneous hypersensitiveness to abortin prepared from five other strains. Guinea-pigs inoculated with heat-killed vaccine or with a mixture of vaccine and kieselguhr did not show cutaneous hypersensitiveness to abortin. Guinea-pigs infected with *B. abortus* gave negative reactions with tuberculin. Tuberculous guinea-pigs gave slight, somewhat atypical reactions with abortin, presumably due to increased nonspecific hypersensitiveness.

AUTHOR'S SUMMARY.

LOCAL SKIN REACTIVITY TO STREPTOCOCCUS HEMOLYTICUS-SCARLATINAE. G. SHWARTZMAN, J. Infect. Dis. **48**:183, 1931.

The skin reactivity was induced by intradermal injection of a potent filtrate of *B. typhosus* "agar washings." The streptococcal reacting factors were present in live cultures of the streptococcus in fluid medium. They were also present, though in lesser concentration, in filtrates of cultures, in toluene-killed cultures and in the supernatant fluid of centrifugated cultures of *S. hemolyticus-scarlatinae*. Reacting factors in stable form were obtained by alcohol precipitation of live cultures. The reacting factors persisted in the material after the removal of bacterial bodies and could be reprecipitated with acid alcohol.

FROM AUTHOR'S SUMMARY.

"ZONING" PHENOMENON IN COMPLEMENT FIXATION. B. S. LEVINE, J. Infect. Dis. **48**:189, 1931.

The results, omitting mathematical formulas, are as follows: In the serum of most adults there are various types of immune substances. On the addition of cholesterized alcoholic beef heart suspension, the action of which is considered nonspecific, interfaces are created at the suspended particles of the colloid. The so-called immune complexes concentrate at such interfaces in spherical and spheroid configuration. With a decrease in the dilution of the serum, the dispersion of the suspended complexes increases, leading to an increase in the surface area of the sensitized spheres in accordance with a special mathematical formula. With a ratio of the dispersion in the serum of the higher dilution to that of the lower exceeding 8:1, the consumption of complement by volume v/2 of full strength serum will be greater than the consumption of complement by volume "v" of full strength serum when both are diluted to the same final volume with saline and the antigen suspension. Hence, "zoning" will appear in all serums in which the consumption of complement by the so-called "nonspecific" antibodies in the first tube of the cold fixation procedure just exceeds, equals or nearly equals one unit.

EDNA DELVES.

DETOXIFYING AND DISINFECTING PROPERTIES OF SODIUM SALICYLATE. K. E. BIRKHAUG, J. Infect. Dis. **48**:212, 1931.

Saturated solution of sodium salicylate neutralizes diphtheria and tetanus toxins in vitro without destroying their antitoxinogenic capacity. Neutralization proceeds in direct proportion to the concentration of sodium salicylate, the duration of contact and the degree of temperature. Simultaneous injection of pure toxin and the amount of sodium salicylate that produces a neutral mixture in vitro fails to avert death from diphtherial toxemia, although the period of survival is prolonged slightly. No neutralizing effect by the saturated solution of sodium salicylate on the toxic filtrates of hemolytic and nonhemolytic was found. This separates these "toxins" from the true exotoxins of diphtheria and tetanus. The bactericidal action of sodium salicylate on the common pathogenic bacteria is about one-tenth that of phenol. The precise mode of action of sodium salicylate in vivo remains obscure. Its dual capacity to exercise antitoxic and antiseptic action in vitro suggests the hypothetic possibility that its therapeutic success in certain infectious diseases is effected by weakening the pathogenic micro-organisms and their capacity to secrete the deleterious exotoxins.

AUTHOR'S SUMMARY.

TUBERCULIN SENSITIVITY OF GUINEA-PIGS UNDER ACIDOTIC DIET. E. H. MICHALOWSKY, Ztschr. f. Tuberk. **59**:321, 1931.

Tuberculous guinea-pigs whose alkali reserve was markedly decreased by an acidotic diet showed weaker skin reactions to tuberculin than control animals under a normal diet and with a normal alkali reserve.

MAX PINNER.

Technical

INOCULATION OF TUBERCULOUS MATERIAL INTO THE LYMPHATIC GANGLIONS. G. NINNI, Ann. de l'Inst. Pasteur **45**:433, 1930.

Rapid diagnosis is secured, particularly with injections of pleural or cerebrospinal fluids, with numerous organisms present in the cervical lymphatic ganglions of the guinea-pig. Results are secured within from eight to twelve days after the injection of small doses. Selective development of the tubercle bacillus, with destruction of other types of organisms that might be present, seems to occur.

M. S. MARSHALL.

COMPLEMENT FIXATION AND THE SERUM LABILITY TEST IN TUBERCULOSIS. G. IVANOVICS, Beitr. z. Klin. d. Tuberk. **75**:657, 1930.

Daranyi's lability test and the determination of the refraction of the serum aid considerably in the diagnosis and prognosis in tuberculosis. They yield constantly positive results in exudative tuberculosis, while they are positive in about two thirds of the fibrotic cases. Complement fixation yields only from 50 to 70 per cent positive results, and it is not strictly specific.

MAX PINNER.

BACTERIOLOGIC DIAGNOSIS OF TUBERCULOSIS. J. MOURIZ, Klin. Wchnschr. **9**:2249, 1930.

The Hohn method is the best for isolating tubercle bacilli from contaminated material. The concentration of sulphuric acid in the technic described by Löwenstein should be reduced to a minimum, but not below from 4 to 5 per cent. The potato-egg medium used afforded an abundant and rapid growth of tubercle bacilli. In order to maintain adequate moisture, a specially constructed tube is recommended.

AUTHOR'S SUMMARY.

THE USE OF ILLUMINATING GAS IN THE PREPARATION OF MUSEUM SPECIMENS. A. SCHULTZ, Klin. Wchnschr. **10**:213, 1931.

The use of Jore's solution saturated with carbon monoxide has been found better for fixing and preserving the color of museum specimens than the previously suggested Kaiserling iodine solution saturated with carbon monoxide.

EDWIN F. HIRSCH.

PREPARATION OF PURE AND STABLE PLASMA FROM MAMMALIAN BLOOD BY CENTRIFUGATION. H. J. FUCHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **69**:305, 1930.

The blood is drawn under special precautions to prevent damage to the platelets and is centrifugated immediately at 14,000 revolutions a minute. The centrifuge is described in *Zeitschrift für Immunitätsforschung und Experimentelle Therapie* (**69**:180, 1930). The secret of success in obtaining stable plasma lies in the complete sedimentation of the platelets before they can disintegrate. Plasma has been obtained by Dilezenne and others according to this principle from birds, reptiles and fishes, with much greater ease because their platelets are not so fragile as the mammalian ones. Fuchs concludes from his work that native plasma does not contain any free prothrombin.

Society Transactions

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Annual Meeting, McGill University, Montreal, April 9, 10 and 11, 1931

SAMUEL R. HAYTHORN, *Vice-President, Presiding*

C. PHILLIP MILLER, JR., *Secretary*

CHARACTER OF THE EXCITATION WAVE IN AURICULAR MUSCLE. FRANK N. WILSON, A. GARRARD MACLEOD (by invitation) and PAUL S. BARKER (by invitation), University of Michigan.

When one electrode of a sensitive galvanometer is placed on the auricular surface and the other on one of the extremities, the resulting curve may be considered a record of the potential variations of the cardiac electrode alone.

The curves obtained from certain auricular regions by this method are, so far as their general outline is concerned, of relatively simple form. The shape of these curves indicates that the electrical disturbances associated with the excitatory process may be represented by a positive pole, or source, just ahead of the crest of the excitation wave, and a negative pole, or sink, just behind it. The approximate interval between the sink and the source may be determined from the experimental curve.

When both electrodes are placed on the heart, one on an injured and the other on an uninjured region, the resulting curve, so far as it is monophasic, is due to potential variations within the injured region.

A theoretical investigation of the electric field surrounding a polarized membrane shows that these observations are in accord with the so-called membrane theory.

NUTRITIONAL ENCEPHALOMALACIA IN CHICKS. ALWIN M. PAPPENHEIMER and MARIANNE GOETTSCH, Columbia University, and the Storrs Experiment Station, Storrs, Conn.

A severe disease of the central nervous system develops in chicks maintained on a diet of milk powder, casein, lard, starch, yeast, salt mixture, cod liver oil and filter paper. Growth is normal until the appearance of symptoms (usually between the eighteenth and the twenty-fifth days). These consist of ataxia, tremors, retraction or twisting of the head, clonic spasms of the legs and stupor. There is found extensive ischemic necrosis of the brain, with hemorrhages and capillary thromboses. The lesions are usually in the cerebellum, less frequently in the cerebrum and rarely in the medulla.

Protective experiments have been carried out with whole wheat, wheat bran, wheat germ, white flour, wheat gluten, wheat ash, wheat germ oil, yellow corn, dried alfalfa, meat scraps, yeast, ether extract of whole wheat, extracted residue, spinach, cabbage and lettuce. Thus far, complete protection has been obtained with none of these substances, but the introduction of whole wheat, spinach and lettuce appears to reduce the incidence of the disease at the levels fed to approximately 20 per cent, as compared with 60 per cent in the untreated controls on diet 108. Toxicity of the cod liver oil can be excluded as a factor. Irradiation of the chicks with the quartz-mercury lamp does not protect. A natural diet composed of a mixture of grains gives protection; this is not affected by autoclaving.

HYPERPLASIA OF THE SKIN OF THE RAT AND MOUSE FROM SULPHYDRYL. STANLEY P. REIMANN, The Lankenau Hospital Research Institute, Philadelphia.

The sulphydryl group (—SH) is the naturally occurring stimulus to cell division. Sulphydryl compounds stimulate mitosis not only in a number of different living forms, but also in a variety of different expressions of cell division such as normal growth, regeneration, repair of wounds, etc. The details and a theory of chemical regulation of growth can be found in the publications of Hammett (*Protoplasma* **11**:382, 1930).

To discover what happens when the sulphydryl group is offered to normal cells, as of the skin, a series of rats and mice was painted with thiocresol in alcoholic solution, in lanolin, in petrolatum and in other vehicles. To the same animals on another part of the body was applied, as control, a corresponding concentration of cresol in the same vehicles.

In about 20 per cent of the animals, when the material was applied in a greasy vehicle, an itching rash developed after about two weeks of three applications weekly. This gradually disappeared over a period of another two weeks, and at the end of six weeks both the animals without the rash and those that had developed it presented the same end-results, namely, a skin obviously thickened and denser to the touch. Histologic examination showed this thickening to be due to a proliferation of epithelial cells until the normal two to four layers of cells in the skin of the mouse and rat became transformed to eight or ten or more layers. The structures preserved their normal relations except for this higher differentiation. The connective tissue beneath also reacted by the production of more cells and greater vascularity. There was, however, no inflammatory exudation in any of the animals. Pathologically, it presented the picture of pure hyperplasia.

In this material, Hammett is studying the type of proliferation, the differentiation of the cells, the differential reactions between the epithelial and the underlying connective tissue, etc., and is preparing reports of his studies for publication.

VARIATIONS IN THE TEMPERATURE OF HEALTHY AND TUBERCULOUS BIRDS. DAVID L. BELDING, Boston University School of Medicine and Evans Memorial.

The study of diseases of birds and the use of birds as experimental animals require a knowledge of the variations in the temperature of normal birds. My observations were made on pheasants, mallard ducks and chickens, and are concerned with the physiologic and pathologic factors that influence the temperature of the body.

The temperature of the body varies with both the species and the individual bird. It increases with age and is slightly higher in the female than in the male. Extreme care is necessary to reduce errors due to the methods of taking the temperature and of handling the birds. Excitement with or without demonstrable muscular activity definitely increases the temperature. This fact throws doubt on the reliability of most of the published records of the temperature of birds.

Birds show a daily rhythm of temperature, the maximum temperature occurring at midday and the minimum at midnight. Exercise produces an initial rise followed by a fall to a subnormal degree, varying in rapidity with species, age, sex and type of exercise. Fatigue and starvation produce a subnormal temperature. Tuberculous birds have a slightly lower temperature than healthy birds.

THE CELLULAR REACTION TO INFECTION WITH TUBERCLE BACILLI; EXPERIMENTS ON THE TESTES OF NORMAL AND IMMUNIZED GUINEA-PIGS. ESMOND R. LONG, ARTHUR J. VORWALD and LILLIAN DONALDSON (all by invitation), the University of Chicago.

Virulent tubercle bacilli of the human type in a dosage of 0.1 mg. per kilogram of weight of the animal were injected into the testes of (group A) normal

guinea-pigs, the testes of (group B) tuberculous guinea-pigs, the locally immunized testes of (group C) guinea-pigs that had received from one to two months previously four intratesticular injections, at weekly intervals, of killed tubercle bacilli, and the previously untreated testis of (group D) guinea-pigs that had received injections of dead tubercle bacilli in one testis. Guinea-pigs of each series were killed after periods of one, three, six, ten, sixteen, twenty-four, forty-eight and seventy-two hours. In each series the great importance of the polymorphonuclear leukocyte was shown. It was the predominant cell in the middle stages of the early reaction in every case, and was highly active in two respects: (1) phagocytosing tubercle bacilli and (2) localizing them by concentration in deep regions of the organ. The speed and intensity of the cellular reaction increased in the different series as follows: (1) the testis of the normal animal, (2) the testis of the tuberculous animal, (3) the nonimmunized testis of the animal with local immunization of the opposite testis and (4) the testis of the animal with local immunization of both with testes. A striking feature was the intense response of polymorphonuclear leukocytes in the locally immunized testis in spite of the fact that large mononuclear leukocytes (monocytes, clasmatocytes, etc.) were already present in large numbers as a result of the immunizing process. In the tuberculous animals exudation of plasma and tubular degeneration were much more intense than in animals of the other series (tuberculin reaction). In each series after from twenty-four to seventy-two hours large mononuclear cells phagocytosed the polymorphonuclear cells containing bacilli, so that after three days the reaction was of the familiar early epithelioid type, the bacilli being seen almost exclusively in large mononuclear exudate cells.

EXPERIMENTAL INFECTION WITH TUBERCULOSIS. ROBERT G. BLOCH (introduced by Russell M. Wilder), the University of Chicago.

An emulsion of virulent tubercle bacilli in saline solution injected intratracheally quickly produces widespread pulmonary tuberculosis, even when very small numbers are injected. It is therefore impossible to produce by this method isolated primary tuberculous infects and complexes. A method was developed to inject intratracheally very small amounts of iodized poppy seed oil 40 per cent loaded with virulent tubercle bacilli and to demonstrate the focus of the iodized oil in the lung by x-ray pictures. On killing the animals after from three to four weeks, the primary tubercle is found where the oil droplet was demonstrated. Comparative microscopic studies indicate that lesions produced in the described way do not differ essentially from the ordinary ones. If they are permitted to develop into more advanced stages, it is found that the oil slightly delays their progress.

CYTOLOGIC REACTIONS IN EXPERIMENTAL MONILIAL ABSCESSES. GEORGE H. ROBINSON and SAMUEL R. HAYTHORN, Singer Research Laboratory, Pittsburgh.

A strain of a pathogenic monilia was used to produce lesions that afforded a study of an exudate containing numerous cells closely resembling the Dorothy Reed cells of Hodgkin's disease.

The lesions produced by the monilia began as small abscesses, which persisted for several days and healed with an unusual type of granulation tissue in which blood vessels were rarely found and in which two strains of mononuclear cells were prominent. One was typical of the ordinary mononuclear phagocytes, and these fused to form foreign body giant cells about moniliae and fibrin. The other type was a large cell with a cartwheel-like nucleus and prominent nucleoli, which were often multiple and which had a slightly granular basophil protoplasm. These cells were found in the walls of the abscess, in the newly formed reticulum and in the lymphoid tissue in nearby lymph nodes, where they were sometimes found in mitosis. The large cells were studied for means of identification in relation to cells of the types constituting reticulo-endothelium, lymphoblastic tissue, early

cells of the bone marrow, or cells of the megakaryocyte series. They were not identified, but their close resemblance to the large cells typical of Hodgkin's disease indicated the danger of diagnosing that disease on the presence of these cells alone.

GROWTH REQUIREMENTS OF GONOCOCCUS IN A LIQUID NONPROTEIN MEDIUM. ALDEN K. BOOR (by invitation) and C. PHILLIP MILLER, JR., the University of Chicago.

In an effort to adapt their protein hydrolysate medium (*Proc. Soc. Exper. Biol. & Med.* **28**:370, 1931) for use in liquid cultures of large volume, the authors encountered certain difficulties that led to the present study. The medium consists essentially of an acid hydrolysate of powdered egg-white, which has been cleared with aluminum hydroxide cream, adjusted to the proper hydrogen ion concentration and buffered, and to which 1 per cent dextrose has been added. It has the advantage of being free from antigenic proteins.

In order to study the effect of varying oxygen tensions, large tubes containing 10 cc. of medium were inoculated with approximately equal numbers of organisms, and the air replaced by mixtures of oxygen, nitrogen and carbon dioxide in known concentration, the percentage of oxygen varying from 10 to 100. The cultures were allowed to stand undisturbed in the incubator. Growth was slow and not very abundant, but it occurred in all tubes and was best in those containing 40 and 60 per cent oxygen, respectively. Varying the carbon dioxide tension seemed to have no influence on the growth when the hydrogen ion concentration was kept constant.

When atmospheric air was bubbled through the inoculated medium, growth was much accelerated, so that at the end of fifteen hours the yield was more abundant than it had been in any of the undisturbed tubes after six days. Agitating a flask of the medium by double eccentric rotation (so that the sides of the flask were being alternately wetted and exposed to the air) resulted in as good growth as was obtained by aeration.

EFFECT OF FILTRATION AND OF VARIOUS CONSTITUENTS OF TISSUE ON THE VIRUS OF POLIOMYELITIS. CLAUS W. JUNGEBLUT, Columbia University.

Titration of suspensions of virus-infected cord of varying percentages permits the finding of a particular concentration with maximum virulence, the concentrations below and above which show progressively diminished virulence.

Berkefeld-N filtrates of suspensions of virus-infected cord are frequently more virulent than the corresponding unfiltered suspensions, suggesting removal of an inhibitory factor from the emulsion.

Spinal cord from a normal monkey added in vitro to filtrates of virus has no appreciable effect on the virulence of the virus. Spinal cord from a convalescent monkey, in one instance, effected a complete inactivation of the infectious agent.

Testicle from a normal monkey added to filtrates of virus in vitro appears to adsorb the greater part of the virus, so that practically all the virus is found in the sediment and very little or none in the supernatant fluid. The testicles of monkeys do not all display this adsorptive effect to the same extent.

Testicle from a normal rabbit is incapable of adsorbing the virus in vitro, but rather tends to enhance the infection.

THE MECHANISM OF THE ACTION OF BACTERIOTROPIN: THE RELATIVE DEGREE OF PHAGOCYTOSIS BY POLYMORPHONUCLEAR LEUKOCYTES AND BY MONOCYTES. BALDUIN LUCKÉ, MAX STRUMIA (by invitation), STUART MUDD and MORTON MCCUTCHEON, the University of Pennsylvania.

In studies on the mechanism of the phagocytosis-promoting action of normal and of immune serums and of their protein fractions, the polymorphonuclear leukocyte has generally been used as the phagocytic cell. The influence on the other principal type of phagocyte, the large monocyte, has received little attention.

The present experiments were designed to compare, quantitatively, the effects of normal and of immune bacteriotropins on the phagocytic behavior of the polymorphonuclear leukocyte and the large monocyte. The relative degree of phagocytosis by these cells was determined toward various kinds of particles (collodion, different strains of acid-fast bacteria, pneumococci, pyogenic cocci, yeasts, etc.).

It was found that:

1. In salt solution alone certain particles are taken up more readily by monocytes than by leukocytes (mammalian tubercle bacilli, collodion particles, etc.).

2. Except under special conditions, deposits of normal or of immune serums on particles causes a parallel increase in phagocytosis by both monocytes and polymorphonuclear leukocytes.

3. The degree of phagocytosis in response to bacteriotropins is, in general, more pronounced with the polymorphonuclear leukocytes than with the monocytes.

IMMUNITY OF TISSUES: CONDITIONS INFLUENCING THE FIXATION AND DESTRUCTION OF BACTERIA IN THE TISSUES. PAUL R. CANNON, E. F. NECKERMANN (by invitation) and F. L. SULLIVAN (by invitation), the University of Chicago.

When approximately equal quantities of bacteria are injected simultaneously into normal rabbits and into others previously immunized, the bacteria steadily leave the blood stream, but at an accelerated rate in the immunized animals. Quantitative studies of organs such as the spleen, liver, bone marrow and lungs indicate that the number of colonies developing per unit volume of tissue is definitely greater, during the first few minutes after injection, in the immunized than in the normal animals. After from thirty minutes to forty-five minutes, however, more colonies per unit volume are present in the tissues of the normal animals.

Histologic studies show that immunization leads to hyperplasia of the splenic follicles as well as of the red pulp, with an increased intensity of phagocytosis in the latter. Furthermore, staphylococci ingested by phagocytes in the red pulp of the immunized animals seem to become swollen and disintegrated more extensively and more rapidly than in the normal control animals. The conclusions are that immunization, by its activation of the mesenchymal tissues, especially in the spleen and liver, elevates the capacity of the tissues to remove bacteria from the blood stream and increases the effectiveness of such tissues in the destruction of the bacteria removed.

THE DEVELOPMENT OF CARCINOMA FROM POLYPI OF THE COLON. H. E. ROBERTSON, the Mayo Clinic, Rochester, Minn.

Studies of the polypi of the colon in various stages of development help to formulate the pattern of their growth and of the stages of transition between them and cancer.

THE PATHOLOGY OF PICK'S DISEASE. CLAUDE S. BECK, Western Reserve University.

The condition designated as "pericarditic pseudocirrhosis" in 1896 and subsequently known as Pick's disease consists of chronic pericarditis as the primary factor and enlargement of the liver, ascites and other signs of circulatory failure as secondary factors. Tuberculosis is generally regarded as the common etiologic factor of the pericarditis, but other forms of infection play a more important rôle. The acute infection usually subsides, leaving only scar formation or deposition of calcium in the pericardium.

The Pick syndrome was produced experimentally by the application of Dakin's solution in the pericardial cavity. The pericardium became scarified. Adhesions between the heart and the pericardium were not necessary to produce the condi-

tion. The essential factor, as shown by roentgenograms, was the contracture of the scar. This impaired the filling of the heart; the venous pressure rose and the minute volume output of the heart decreased. Ascites and enlargement of the liver developed. The surface of the liver showed a deposition of fibrin and fibrous tissue, and this finding indicated that the classic "Zuckergussleber" was not necessarily due to infection. Hydrothorax and subcutaneous edema developed late and were terminal manifestations. The electrocardiograms showed slurring and decreased voltage.

The method provides material for investigation by both clinician and surgeon.

A Spectrophotometric Study of Blood Solutions. R. P. Kennedy, the University of Rochester.

Some observations on solutions of blood that correlated values for oxygen capacity with quantitative measurements of color were published in an earlier paper (*Am. J. Physiol.* **79**:346, 1927). Certain optical constants were also evaluated for oxyhemoglobin. The data included here represent a similar spectrophotometric study of blood pigment in its reduced form hemoglobin. Spectrophotometric data of this kind have been available in the literature for a long time, but these were derived from the measurement of the color of blood reduced by chemical means or reduced by allowing it to putrefy (Hari, P.: *Biochem. Ztschr.* **115**:54, 1921). It seems desirable, therefore, to gather additional information concerning the change in the color of solutions of blood when they are simply subjected to reduced oxygen pressure. Also, it is important to ascertain whether or not the color returns to the original in the reverse reaction. In this work the change in color from the oxidized to the reduced form was studied in three steps: First, a spectrophotometric curve was plotted for a solution of oxyhemoglobin. Second, the oxygen was removed by diminished pressure and the corresponding color recorded. Third, the same solution was resaturated with air and a second oxyhemoglobin curve made and compared with the first. Thus, the reversible reaction of oxygen and hemoglobin may be studied from the standpoint of colorimetry.

In addition to the examination of the color of dilute solutions of blood, extracts were made of skeletal muscle of perfused dogs and similar spectrophotometric measurements made on these extracts in both the oxidized and the reduced forms. It is interesting to compare the shape of spectrophotometric curves of blood pigment with that of such curves obtained from blood-free skeletal muscle. Only slight differences are observed, and these are discussed. A comparison of the curves of oxidized and reduced pigment opens up the interesting possibility of determining an oxygen dissociation curve for hemoglobin in dilute solutions.

The Significance of Water Balance in Epilepsy. F. B. Byrom (introduced by Russell M. Wilder), the University of Chicago.

The water balance in epileptic patients was determined by the method of Newburgh.

The ketogenic diet causes an immediate loss of body water, equilibrium then being established at a lower level. Two factors are mainly responsible: First, the removal of protein-sparing carbohydrate from the diet causes a temporarily negative nitrogen balance and a proportionate liberation of water held in physical combination. Second, a loss of fixed base (sodium) occurs, which is also arrested after a few days by mobilization of the urea-ammonia mechanism. The individual convulsions also cause a loss of water. Further attacks may, however, occur during this negative phase, while the reactionary phase of water retention that follows may be free from attacks. It is concluded that the convulsions in epilepsy cannot be explained solely on the basis of disturbed water balance.

RELATION OF SULPHUR TO EXPERIMENTAL ANEMIA. O. M. GRUHZIT, Parke, Davis & Company, Detroit.

A severe hemolytic anemia was induced in dogs by feeding onions, onion fractions and some disulphide derivatives of the general formula R-S-S-R, which are chemically closely related to oil of onions, the allyl disulphide. The work on disulphides was followed by a similar study of monosulphides of the general formula R-SH. The radical "R" in these compounds represents an aliphatic or aromatic group. This study covered propyl, tolyl, benzyl, diphenyl disulphides, l-cystine and dithioglycollic acid. In the series of monosulphides were included thiophenol, thiocresol and butylisothiourea. Since some of these compounds are sulphur homologues of phenol, the latter was similarly studied.

In these series feeding of the benzyl disulphide, cystine, dithioglycollic acid, butylisothiourea and phenol caused no anemia in dogs. The feeding of propyl, tolyl, diphenyl disulphides and the monosulphides, thiophenol and thiocresol, caused severe acute hemolytic anemia. The maximum hemolytic effect was reached in from five to seven days. The hemoglobin dropped to less than 20 per cent by Sahli, the number of the red cells decreased to less than 1,000,000 per cubic millimeter. The white cell count rose to 60,000 per cubic millimeter. The reticulocytes at times constituted 85 per cent of the total red cells. The red cells showed various degenerative forms, and microscopically it was difficult to differentiate the blood picture from that of pernicious anemia. The color index also approached 1 and at times it was over 1. The leukocyte and reticulocyte counts always remained high and increased in proportion to the severity of the anemia. At no time was I able to induce aplasia of the bone marrow. In some of the dogs the hemoglobin has been kept at a level around 20 per cent (by Sahli) for the last six months without difficulty, and no tolerance has developed, except to diphenyl disulphide and probably to the monosulphides tested. Discontinuation of the feeding of sulphide reverted the condition of the blood to normal.

The anemia caused by the feeding of sulphide is probably of the same general type as that induced by compounds of the type of phenyl hydrazine. The hemolytic action of compounds of the type of sulphide is probably related to a fixed stability of sulphur to the radical group. In benzyl disulphide the linkage of the sulphur to the radical is unstable, the compound undergoes rapid degeneration, and no anemia was induced by this compound. The tolyl disulphide, an isomer of benzyl disulphide with sulphur in stable relation to the radical, possessed high hemolytic action. The thiophenol, the sulphur homologue of phenol, possessed extremely high hemolytic activity, yet phenol had no hemolytic action. In cystine, thioglycollic acid and butylisothiourea the sulphur is bound weakly to the radical. These compounds were devoid of hemolytic properties.

In undertaking this study I was interested to develop a method for testing the potency of various substances used in the treatment for pernicious anemia. This phase has not been as yet thoroughly exhausted, but evidence has accumulated to show that desiccated hog stomach or potent liver extracts counteract the hemolytic action of sulphides. The hemoglobin values and the red cell counts begin to increase and the white cell counts and those of the reticulocytes to decrease after a simultaneous maintenance of dogs on the disulphide and the anti-anemic extracts. This did not occur as definitely and as regularly when the animals were maintained on extract devoid of activity or when inert liver ash was substituted for a potent extract.

NUTRITIONAL MUSCULAR DYSTROPHY IN GUINEA-PIGS AND RABBITS. MARIANNE GOETTSCH and ALWIN M. PAPPENHEIMER, Columbia University.

An extensive and ultimately fatal dystrophy of the voluntary muscles was induced in guinea-pigs and rabbits by dietary means. The young animals at the time of weaning were given a natural diet, consisting of wheat bran, rolled oats, casein, skimmed milk powder, lard, cod liver oil, sodium chloride and calcium

carbonate, in which vitamin E had been destroyed by treatment with ethereal ferric chloride, and 3 or 6 cc. of orange juice daily.

Later experiments showed that the deficiency of vitamin E was not responsible for the disorder. Neither was the treatment with ethereal ferric chloride a factor. Scurvy, inanition and infection could be ruled out. Control animals on a diet of bran, oats, hay and greens had normal muscles. Rats were not susceptible to this dietary disease.

THE ACTION OF EPINEPHRINE IN DEPANCREATIZED ANIMALS. JESSE L. BOLLMAN, the Mayo Foundation, Rochester, Minn.

Depancreatized animals were maintained in good nutritive condition by large doses of insulin and appropriate feeding. Four days after the withdrawal of insulin, and fasting, specimens of muscle and liver were removed under ether anesthesia. Large amounts of epinephrine were then given over a period of twelve hours, at the end of which additional samples of liver and muscle were taken. The urine during the administration of epinephrine showed an excess of sugar that could not be accounted for by the nitrogen excreted, and a small amount of lactic acid was excreted. The glycogen content of the liver showed little change, and an increase in dextrose content was usually noted. The dextrose content of the muscle showed few changes, but the glycogen content of the muscle was reduced during the administration of epinephrine to very low values (from 20 to 40 mg. for each 100 Gm. of muscle). In all experiments the dextrose excreted in excess of D/N 2.8 was almost identical in amount with the loss of glycogen from the muscle. No evidence of the formation of dextrose from other substances (fat) was obtained.

MENSTRUATION AND FOCAL REACTIONS. W. F. PETERSEN, LLOYD ARNOLD and MARGARET MILLIKEN (by invitation), the University of Illinois.

Focal reactions in areas of either acute or chronic inflammation or in areas the seat merely of cellular dysfunction commonly present clinical manifestations. Indeed, not infrequently the entire clinical picture is dominated by the focal reaction.

The biologic alterations associated with the menstrual cycle are apparently frequently associated with the inception of such focal reactions, which may become manifest in the skin or mucous membranes, in the internal organs, in the central nervous system, etc.

There is a further importance that attaches to focal reactions inasmuch as we make use of them therapeutically in nonspecific therapy, and they probably play a rôle in the normal processes of healing and recovery, particularly in chronic infections. It is for this reason that the authors have studied in considerable detail the chemical alterations that occur during the course of the menstrual cycle and present a series of charts in which correlating observations have been made on the emotional status of the patient, the disinfecting power of the skin, the blood calcium, potassium, chlorides, sugar, cholesterol, phosphates, carbon dioxide content, hydrogen ion concentration, protein content, the leukocyte count, as well as basal metabolism, and such manifestations of the skin as the intracutaneous reaction to histamine and epinephrine, the dermographic reaction time, blister permeability, etc.

For the focal reaction that occurs in tuberculosis, the importance of the diminution in cholesterol, of the deficit of alkali and of the great increase in permeability of the capillaries that occur in the premenstrual phase would appear to be of considerable significance. Presumably the premenstrual phase is unfavorable so far as resistance to tuberculosis is concerned, while the chemical conditions that obtain in the postmenstrual period seem to be favorable toward healing and recovery.

For a syphilitic infection the opposite is probably true.

THE AVAILABILITY OF THE CALCIUM RESERVE OF THE SPONGY TRABECULAE AND COMPACT BONE IN EXPERIMENTAL HYPERPARATHYROIDISM. HENRY L. JAFFE, AARON BODANSKY and JOHN E. BLAIR (by invitation), Hospital for Joint Diseases, New York.

We have shown that young and adult guinea-pigs are susceptible to the effects of single and repeated doses of parathormone; the adult guinea-pigs show smaller changes in serum calcium and phosphorus and less resorption of bone than the younger animals. Histologic studies of the bones of guinea-pigs with acute or chronic experimental hyperparathyroidism have brought out the fact that the spongy trabeculae of the epiphyses are the least susceptible to decalcification. We have been unable to confirm the conclusions of Bauer, Aub and Albright that the bone trabeculae are more easily depleted than the cortex and are the source of labile calcium reserve. In young animals before closure of the epiphyseal cartilage plates the greatest degree of decalcification occurs in the metaphyses, at the costochondral junctions, and in the cortices of the ribs and long tubular bones. In older or in adult guinea-pigs the diaphyseal cortex is most involved. The trabeculae of the epiphyses showed no evidence of resorption in any of our animals except in one adult guinea-pig given 2,580 units of parathormone in doses pyramided over a period of forty-eight hours.

RENAL DENERVATION: I. EFFECT OF SNAKE VENOM. II. EFFECT OF REPEATED CHILLINGS. G. MILLES, E. F. MÜLLER and W. F. PETERSEN, the University of Illinois.

Accurate delineation of the renal vascular bed was obtained by the antemortem injection of 25 per cent solution of bismuth oxychloride into the abdominal aorta above the origin of the renal arteries. The kidneys were then removed, and x-ray photographs of them were made.

When this method was applied to animals on which unilateral renal denervation had been performed two or more weeks before, it was found that the injection of snake venom caused much more profound changes in the denervated than in the normal renal vascular tree, as demonstrated by immediate injection of the kidneys. From this it is concluded that the innervation of the kidney, by vasoconstriction, protects the organ from exogenous toxins.

On applying the same method for the demonstration of the renal vascular bed, it was found that daily chilling repeated for periods up to six months resulted in a marked decrease in the size of the vascular bed in the normal kidney as compared with the denervated kidney. The conclusion is drawn from this that vasoconstriction of the kidney is obtained by chilling and when repeated over a period of time results in definite vascular damage.

THE BONE MARROW IN AGRANULOCYTOSIS. RAPHAEL ISAACS, the University of Michigan.

In a patient who died from a severe infection with necrosis of the skin of the scrotal and perineal region, the blood showed a practically complete disappearance

	Sternal Marrow, per Cubic Millimeter	Vertebral Marrow, per Cubic Millimeter
Total cells	1,062,000	834,000
Red blood cells	666,000	504,000
Nucleated cells (all types)	396,000	330,000
Polymorphonuclear cells	0	0
Metamyelocytes	0	0
Myelocytes	0	0
Myeloblasts	0	0
Eosinophils ("lymphoid")	29,526	37,635
Endothelial cells	62,526	46,315
"Lymphoid" cells	255,316	205,525
Erythroblasts	48,632	40,525
Megakaryocytes	Present	Present

of polymorphonuclear neutrophil leukocytes. On the day of death the white blood cell count was 700 per cubic millimeter. During the four weeks of illness the febrile reaction was marked, the temperature ranging from 102 to 104.5 F. The

bone marrow from the sternum and a vertebra, taken at autopsy, was very red, and the bone trabeculae were prominent. The cells were counted by drawing up fresh marrow to the "5" mark of a Sahli hemoglobin pipet, and diluting twelve times with blood serum and further diluting with Hayem's solution as in making a red blood cell count. The nucleated cells and red blood cells were enumerated separately. The remaining suspension of cells and serum was used to make cover glass films for differential cell counts (Wright's stain). The table shows the quantitative data.

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, April 13, 1931

JOSEPH A. CAPPS, *President, in the Chair*

UREMIC DERMATITIS. S. R. ROSENTHAL.

The characteristic macroscopic change of the skin in uremia is a "uremic frost." This occurred in thirty-three of fifty-six patients with uremia. In forty cases in which the skin was studied histologically there was a gradual atrophy of the skin directly proportional to the duration of the disease. This atrophy is explained on the basis of a constant saturation of the skin with urea (determined by the xanthydrol reaction), which interferes with the afferent and efferent circulations. Urea is not toxic, and it is found in the skin in direct proportion to the concentration of urea in the blood.

This complete report appears in *Arch. Dermat. and Syph.* **23**:934 (May) 1931.

THE EARLY CELLULAR REACTION TO HUMAN TYPE TUBERCLE BACILLI IN THE LUNG OF THE RABBIT. ARTHUR J. VORWALD.

A previous study of the histopathologic reaction of the tissue of the lung in normal animals of varied species, at intervals of one day, one week and one month after an intravenous injection of a known amount of human type tubercle bacilli, demonstrated that in order to differentiate cell types partaking in the reaction intervals of less than twenty-four hours should be studied. My purpose is to describe the cellular response occurring in the lungs of normal rabbits at intervals of from one hour to one month after inoculation, stressing particularly the rôle of the polymorphonuclear leukocyte.

Rabbits were given intravenous injections of 0.1 mg. of human type tubercle bacilli per kilogram of body weight. The animals were killed at selected intervals after inoculation, and the lungs were immediately fixed in situ by the intratracheal injection of Zenker's solution of formaldehyde. The tissues were embedded and sectioned in celloidin. The tubercle bacilli were stained with carbol fuchsin, and for counter stain hematoxylin eosin-azure was used in one series and hematoxylin g-azure in another.

During the earliest stages, the first cells to react against the tubercle bacilli are the polymorphonuclear leukocytes, which encompass and phagocytose the organisms. Such foci invariably contain bacilli. This reaction is confined at first to the small capillaries, and then extends to the adjoining alveolar walls and spaces. Within fourteen hours, the polymorphonuclear leukocytes with and without tubercle bacilli are phagocytosed extensively by the mononuclear exudate cells, which increase in number to the exclusion of the leukocytes. The tubercle bacilli thus are liberated in the cytoplasm of the exudate cell. At stages later than eighteen hours, the mononuclear exudate cells with and without bacilli dominate the reaction so that at one month they constitute by far the majority of the cells in the typical tubercle. At this stage most of the mononuclear exudate cells have an oval, vesicular nucleus and a large, slightly foamy, pale blue cytoplasm. There is no selective localization of tubercle bacilli in the lymphoid aggregates. Almost always the bacilli lodge in the alveolar walls and there set up an inflammatory reaction.

Throughout all stages the absence of mitotic figures in the cellular reactions is striking.

I have reached the following conclusions:

The earliest cells responding to an intravenous injection of tubercle bacilli in rabbits are the polymorphonuclear leukocytes.

This reaction is inflammatory and specific. The polymorphonuclear leukocytes phagocytose the tubercle bacilli and play a great rôle in the early localization of these organisms.

Mononuclear exudate cells later dominate the cellular response, phagocytose and digest the polymorphonuclear cells and engulf their contained tubercle bacilli.

There is no primary localization of tubercle bacilli in the lymphoid aggregates of the rabbit's lung.

HEMORRHAGE AND "SHOCK" IN TRAUMATIZED LIMBS: TOTAL, FREE AND BOUND WATER CHANGES OF BLOOD AND MUSCLE. WILLIAM ROBINSON and ELOISE PARSONS.

The water content of the blood and muscles, in respect to both total water and water in the "free" and bound state, has been studied in animals under the influence of hemorrhage, histamine and trauma. The free water is that which freezes at —20 C., as determined by the "heat of fusion of ice" method of Rubner. The total water is determined by drying the material until a constant weight is obtained. The difference is termed bound water. The method is such that small samples can be analyzed, so that frequent determinations during the course of an experiment can be made and changing conditions can be studied.

The observations show that with hemorrhage there is a compensatory transfer of water chiefly in the "free" state from the muscles to the blood. In "histamine shock" the fall of blood pressure is associated with, and perhaps the result of, a transfer of water from the blood to the muscles. In "shock" produced by severe trauma to the leg (produced by blows from a padded hammer), the evidence indicates that with minor trauma there is in some cases a loss of fluid from the blood to the muscles associated with a slight fall in blood pressure, and that with major trauma the changes are those of hemorrhage in that there is a dilution of the blood remaining in the general circulation, at the expense of water withdrawn from the muscles.

The findings in this study are in support of the work of Parsons and Phemister, Blalock and others, which indicates the importance of the loss of blood in traumatized tissue in causing the fall of blood pressure after severe trauma.

SQUAMOUS CELL CARCINOMA IN A DERMOID CYST OF THE OVARY. P. A. DELANEY.

Dermoid cysts constitute about 10 per cent of all ovarian tumors, and in about 1 per cent malignant changes occur, usually carcinoma. In 1929, Masson and Ochsenshirt (*Surg. Gynec. Obst.* **48**:702, 1929) reported three dermoid cysts with squamous cell carcinoma from the Mayo Clinic, their contribution bringing the total to thirty-six.

A white woman, aged 60, entered the Englewood Hospital, Chicago, because of pelvic pain of six or seven years' duration, which had become progressively worse especially during the three months before admission. On physical examination, a pelvic tumor was found. Operation revealed a large cyst containing about 1 quart of creamy fluid and large amounts of hair. The base of the cyst was adherent to unrecognized structures deep in the pelvis and consisted of friable tissue that appeared malignant. The abdominal wound never completely healed, and a fistulous opening persisted until the patient's death nine months later with symptoms of recurrence of the tumor. She died away from the city, and autopsy was not performed.

The emptied cyst weighed 156 Gm. The wall of the cyst, away from the base, where it was from 2 to 3 mm. thick, had a stratified squamous epithelial lining with frequent transitions to pseudostratified and simple columnar, partly

ciliated epithelium. Among the simple columnar epithelium were theca cells with specific mucin reactions. The subepithelial connective tissue stroma contained hair follicles with sebaceous glands, sweat glands and smooth muscle cells. Tissue from the base of the cyst showed extensive accumulations of anaplastic squamous epithelium with frequent "pearl" formation. Numerous large areas of necrosis, as well as many giant cells, involved both the stroma and the malignant tumor. In some of the sections with extensive carcinoma, cortical ovarian tissue stroma was identified. Sections removed from the inner lining of the cyst at its base, near where ulceration was present, had a gradual transition from normal to malignant, stratified, squamous epithelium.

MIXED TUMOR OF THE SCALP. P. A. DELANEY.

In 1930, Dr. Erb of the Konigsberg Policlinic published a lengthy article entitled "Rare Mixed Tumors of the Scalp" (*Beitr. z. klin. Chir.* **149**:617, 1930). He reported the first one seen in his clinic and contributed the fifth to the literature. His bibliography contains no reference to American, English or French authors.

A white man, aged 59, went to his physician for the removal of a small tumor over the left parietal prominence. He said that in 1915 or 1916 he had bruised the scalp. The injury healed without a scar, but about six months after the accident a barely perceptible mass appeared which grew slowly but progressively. Surgical removal was sought largely for cosmetic reasons.

A preoperative diagnosis of sebaceous cyst was made, but a solid tumor closely adherent to the overlying scalp was found. The surgical wound bled profusely and was closed without assurance that all of the tumor had been removed. The part removed was firm, white and yellow tissue weighing 1 Gm. and was 15 by 10 by 7 mm. It was fixed in Bouin's fluid.

Histologic preparations stained by various methods had a narrow fibrous connective tissue capsule. Epithelial tissues as intercommunicating and free tubuloalveolar structures formed most of the section and frequently had a lumen content of individual round to oval cells that resembled those of the layers of the inner wall, their cytoplasm being homogeneous and acidophilic; the basal cell layer or layers had an intracellular content of fibrillae similar to that of the excretory duct of related marginal groups of sweat glands, or that of so-called myoepithelial cells common to the sweat and mammary glands. In many places there was a proliferation of basal fibrillar cells into the adjoining stroma, while in other locations the inner nonfibrillar cells had proliferated and invaded the stroma to induce marked changes and become greatly altered in structure. The stroma was homogeneous and stained intensely red with mucicarmine, while most of the cells were polymorphic and vacuolated, singly or in groups. No daughter capsules were evident, but the stroma about the isolated or grouped cells frequently took a deeper stain, giving the impression of a capsule. On superficial examination, this latter tissue had cartilaginous characters, but in fact it was stroma tissue with changes accompanying the advance of invading epithelial cells of a specific type. The tumor did not have stellate myxoblasts. There were small isolated areas resembling intracanalicular fibro-adenoma of the breast and groups of tumor cells in the lumen of blood vessels without connection with the endothelial lining. There was no mitotic activity.

Four months after operation, the patient had a healed surgical scar and two small subcutaneous masses about 2 and 4 mm. in diameter, respectively. Five or six hair shafts projected over them, although the patient had an advanced stage of baldness.

This tumor illustrates the transformation of collagenic connective tissue stroma into a pseudocartilaginous matrix by the invasion of epithelial cells. This suggests that a similar change takes place in mixed tumors of the salivary glands. Although the structure of the adenoma favors origin in sudoriparous glands, the possibility of the participation of sebaceous glands or hair follicles in its formation is not disproved.

THE ASSOCIATION OF PSEUDOHERMAPHRODITISMUS MASCULINUS EXTERNUS WITH ADRENAL HYPERPLASIA AND VASCULAR HYPERTENSION. CLAIRE E. HEALEY and CHESTER C. GUY.

This article will be published in full in a later issue of the ARCHIVES.

Regular Monthly Meeting, May 11, 1931

PAUL R. CANNON, *President pro tem., in the Chair*

THE IMMUNOLOGIC SPECIFICITY OF ANTIGENS OF THE BRAIN. JULIAN H. LEWIS.

Many attempts have been made to demonstrate for the various organs of the body an immunologic specificity that is comparable to the specific character of their functions, anatomy and chemical composition. The existence of organ-specific antigens permits the immunologic differentiation of the different organs within the same species and establishes a relationship of the same organ in different species. The classic example of such an organ specificity is that of the crystalline lens. An absolute differentiation of this organ from all other organs in the body can be made by appropriate immunologic reactions, and as a corollary, no differentiation can be made between the crystalline lens of any species. This complete organ specificity also carries with it the ability to form iso-antibodies. An animal can be sensitized and shocked with its own lens. As to other organs of the body, a slight and inconstant differentiation can be made within the species. This has been found to be particularly true in comparisons of the sex organs with other organs, but no relation has been found between sex organs of different species.

Recently, Witebsky reported that the brain can be placed in the same class as the crystalline lens, since it contains antigens that show absolute organ specificity. The only difference between the crystalline lens and the brain is that the specific antigen of the latter is reported to be lipin. Because of the importance of this contribution, I have repeated and extended Witebsky's experiments in order to verify them.

Rabbits immunized with a suspension of rat, beef or guinea-pig brain form antiserums that react not only with suspensions of brains of other mammals, but also with those of birds, fish and reptiles. Alcoholic extracts serve as antigens just as well as do the suspensions. By saturation experiments, some differentiation can be made between these interacting antigens of the brain, since by saturation experiments all antibodies for a heterologous antigen can be removed without removing the antibodies for the homologous antigen of the brain.

GAUCHER'S DISEASE. ERNEST KRAFT and HENRY F. HOOKER.

An unmarried woman, aged 25, with a markedly enlarged spleen, had lost weight and strength for several months. The spleen, which was removed surgically, measured 31.2 by 21.6 by 14.4 cm. and weighed 6,800 Gm. The histologic structure was that of Gaucher's disease. Following splenectomy, the patient improved markedly in health. The father and a brother of the patient are known to have had enlargement of the spleen.

CONGENITAL DIAPHRAGMATIC HERNIA ASSOCIATED WITH AN ACCESSORY LUNG. CHESTER C. GUY and GEORGE RAND.

A full-term female infant lived one hour after birth. A diaphragmatic hernia on the left bulged into the chest and occupied three fourths of the left half and contained the stomach, the small bowel, the cecum, the ascending colon, the pancreas and the spleen. In the lower portion of the left pleural cavity, behind and medial to the hernial sac, was an accessory mass of lung tissue, 4 by 2 by

2.5 cm., attached medially to the upper surface of the diaphragm at the edge of the hernial opening by a pedicle 8 by 8 mm. The arterial blood supply was chiefly through a branch from the thoracic portion of the aorta. Microscopically, the structure of the tissue was that of fetal tissues of the lung with atelectasis.

FAMILIAL CONGENITAL ATRESIA OF THE INTESTINE. ERIC A. FENNEL.

A secundipara, aged 27, was delivered of a male child with a distended abdomen. The anal opening of the child was patent, but an obstruction of the rectum was found 2 inches (5 cm.) above. Eight hours after birth, with the patient under spinal anesthesia, laparotomy released more than a pint of serofibrinous exudate and demonstrated extensive adhesions between the abdominal viscera. The rectum was small, and the sigmoid colon had a diameter of 9 mm. and seemed solid. A tube was inserted into the rudimentary lumen, and the laparotomy wound was closed. The child died seven hours later (fifteen hours after birth). Cultures of the peritoneal exudate were sterile. There were no changes in the esophagus, lungs, heart, pleurae, gallbladder, bile ducts, kidneys, ureters and urinary bladder. A generalized fibrinous exudate covered the viscera of the abdomen. The lower portion of the duodenum had a thin wall and an empty dilated lumen. The jejunum and ileum were distended with meconium, soft in the upper loops and firm in the lower loops. The wall of the small bowel was thin. The cecum was large; the colon was firm and had a diameter of 9 mm. The wall of the first 7 cm. was thin; the lumen was 7 mm. in diameter and contained brown meconium. Beyond this portion, the lumen of the colon was a small cleft which disappeared in the rectum. Histologic preparations of the small bowel had no noteworthy changes. Those of the colon had the usual tissue structures. There were no inflammatory reactions.

On the third day after birth, the brother of this child, who was born to the mother thirteen months before her second confinement, had symptoms suggesting pyloric stenosis. The distal 8 inches (20.32 cm.) of the small bowel and the entire colon had a diameter of 1 cm., and the cecum was small. There were no tortions or thrombosed vessels. No congenital anomalies are known in the families of the father and the mother of these infants. Some hereditary factor seems concerned with the malformations.

Book Reviews

DIE SEXUELLEN ZWISCHENSTUFEN. By RICHARD GOLDSCHMIDT, Professor und Direktor am Kaiser Wilhelm-Institut für Biologie im Berlin. Price, 45 marks. Pp. 520, with 214 illustrations. Berlin: Julius Springer, 1931.

The problem of the unusual conditions of the reproductive system, which are of far more frequent occurrence than is known by even the most scientific investigators, has at once a remarkable attraction for the general scientist, biologist, animal breeder and clinician. The once generally accepted idea of the stability of sex and its concreteness — entire maleness or femaleness — is gradually giving way to an appreciation of the plasticity of sex, in extreme cases of which an organism may at one time display the female characters and produce ova and at a later time develop the characters and psyche of the male and even produce spermatozoa. Between the two extremes of maleness and femaleness there exists a vast field of sexual intermixtures — intersexes, sex intergrades, hermaphrodites and gynandromorphs — in which all stages of the reproductive organs and associated external and internal structures of both sexes are represented in the same organism.

Professor Goldschmidt is a leading authority on the subject of intersexuality by virtue of practically continuous investigation in this field for more than twenty years. In this book he not only summarizes his own extensive investigations dealing with the crossing of races, embryology, cytology and genetics as they have been means of analyzing the sexual conditions presented, but also gives an exhaustive review of the facts and theories of intersexuality presented by other investigators and covering the entire animal kingdom.

After carefully distinguishing between such types of sex intermixtures as the true intersexes and gynandromorphs Goldschimdt proceeds to analyze the approach to the study of the problems and properly emphasizes the necessity of knowing the embryologic, anatomic, cytologic and genetic phases of the particular individual or group under investigation. His own extensive contributions on the peculiarities that have arisen in *Lymantria,* the gypsy moth, in which for example a given male when crossed with female (a) of one race produces offspring that are normal, whereas when crossed with female (b) from another race produces offspring all, or definite proportions, of which show reproductive systems or other sex characters of mixed type and covering the complete range from one sex to the other, are explained by different intensities, valences or strength of the individual sex determiners. Thus a race may be out of balance in its sex determiners with a different race, and the progeny arising with the imbalance of determiners for maleness or femaleness may show an intermediate or mixed development that may occur at different critical periods in development. The relative period with which the imbalance becomes effective in development accounts for the different degrees of intermixture. A zygotically determined female offspring, for example, may show this critical point sufficiently early in development as to develop completely into a male and so function. Genetic analyses have revealed the true nature of such offspring. Following the impressive array of evidence in support of his quantitative theory of sex, now generally recognized as a fundamental biologic principle in the determination of sex, Goldschmidt reviews the known facts of intersexuality demonstrated among fish, amphibia, birds and mammals, including man; he thus offers a common ground for an explanation of intersexuality among all the animal groups, invertebrates as well as vertebrates, but as sharply as possible he distinguishes between genetic and hormonal influences as they operate in the life of the organism.

The reader is impressed with the general organization and the handing of the vast complexities brought under consideration. Much more than half of the book deals with intersexual conditions found among the vertebrate group. The book is excellently illustrated with both original and recently published illustrations and with instructive explanatory diagrams. It is notable that a great deal of the literature of 1930 forms an integral part of the book, hence it is well up to date on the findings in this particularly interesting field of research.

THE PHYSICIAN OF THE DANCE OF DEATH. A HISTORICAL STUDY OF THE EVOLUTION OF THE DANCE OF DEATH MYTHUS IN ART. By ALDRED SCOTT WARTHIN, PH.D., M.D., LL.D., Professor of Pathology and Director of the Pathological Laboratories in the University of Michigan, Ann Arbor, Mich. Price, $7.50. Pp. 142, with 92 illustrations. New York: Paul B. Hoeber, Inc., 1931.

This book is a reprint with additions and corrections of articles in *Annals of Medical History*. Since his student days the author has felt a special interest in representations in art of human death. During the years scattered hours of leisure were given to the study of writings and works of art representing the thoughts about death by European peoples during the past six centuries. Little by little the author gathered a unique bibliographic collection concerning the Dance of Death, and this collection is the basis of the book.

The physician in the Dance of Death motive, in which the personified human skeleton stands for the process of death, is followed from the late fourteenth century to the present time in scholarly and artistic discussion. The introduction deals with the beginnings and early significance of the Dance of Death. The discussion of its further evolution is divided logically as follows: The period of the great wall paintings; the pre-Holbein manuscripts, block books and the Incunabula; Holbein; the imitators of Holbein; the period of caricature; the modern Dance of Death. Most of the illustrations reproduce various pictures of Death and the Physician. The physicians of the past are seen under conditions that reflect their ways of practice, their character and social standing and the contemporary state of medical knowledge. The anatomist is represented in these figures, but the pathologist seems to have been overlooked. At present, the physician himself seems to be stepping out of the picture.

"The Dance of Death idea is as immortal as the life of the race; but in each new period of human thought it will express itself in new form corresponding to the predominant philosophy of that time. From the primitive wall paintings of the Middle Ages as marking the period of religious superstition, to the intellectual creation of Holbein as characterizing the Renaissance, and following this the gradual decrescendo through the Rococo period to modern times, the Dance of Death motive has followed a definite evolution which now awaits the artist of the modern period, who will express adequately in some art form the changed philosophy of the twentieth century toward Death; the Totentanz mythus awaits a new birth."

The work is a choice product of the wise use of leisure hours in the pursuit of a fascinating hobby.

ANTIGENS IN THE LIGHT OF RECENT INVESTIGATIONS. By OLUF THOMSEN, Professor of General Pathology in the University of Copenhagen. Translated from the Danish by Hans Andersen, M.D. Pp. 187. Copenhagen, Denmark: Levin and Munksgaard, 1931.

The book is divided into three chapters. The first chapter deals with the definition of antigens and antigenic functions. In the second chapter, which is the longest, the different kinds of substances that may act as antigens are discussed. First come the proteins, the natural as well as the artificially altered;

then the lipoids, including the heterogenetic antigens, the carbohydrates and their rôle in the specificness of bacterial species and bacterial types; the so-called chemospecific antigens, and finally, the true bacterial toxins. The third chapter considers certain special problems in the light of the progress in the study of antigens: the nature of the Wassermann reaction, lipoid anaphylaxis, idiosyncrasy (synonym for hypersusceptibility, sensitization and allergy) and bacterial allergies.

The author has succeeded well in presenting the main advances of the recent investigations of antigens. The book will be particularly helpful in reviewing the present knowledge of haptenes, carbohydrates, lipoids and chemospecific antigenic substances and their rôle in immune reactions, in specificness and in allergy. The advances in the understanding of the rôle of haptenes may lead to a more satisfactory interpretation of certain forms of hypersusceptibility. Technical details of the preparation of antigenic substances or of tests of various kinds are not discussed. The great problem of how antigens act in the body in the production of antibodies is hardly touched. But then it is true that no signal advances have been made in respect to the fundamental problem of the production of antibodies since 1920, which is the period with which the book is particularly concerned. The course and distribution of antibodies in the immunized organism are not considered.

The translation is fairly satisfactory; in the main the meaning is clear, but at times the language is cumbersome and occasionally one notices peculiar turns, e. g., "regarded after this theory" in place of interpreted in accord with this theory; in other instances the word after is used in the sense of because; "the hypothesis seems constructed" in place of forced or labored. In the section on idiosyncrasy (allergy, hypersusceptibility), idiosyncratic is used freely as synonymous with allergic, hypersusceptible and hypersensitive. An index would have been helpful. The German word "Schlepper," which is used by Sachs and others to describe the more or less mechanical rôle of foreign serum in establishing the antigenic effect in vivo ascribed to certain lipoids, has not received a satisfactory English equivalent. The suggestion is ventured that "conveyor" or "carrier" seems to meet the need. The general quality of the subject matter of the review is praiseworthy. The topics presented are discussed satisfactorily in a critical but constructive and helpful spirit.

THE FACTOR OF INFECTION IN THE RHEUMATIC STATE. By ALVIN F. COBURN, Resident Physician of the Presbyterian Hospital in the City of New York. Price, $6. Pp. 288, with 48 illustrations. Baltimore: Williams & Wilkins Company, 1930.

Most of the material for this book was obtained from a group of 162 patients with rheumatic fever and rheumatic endocarditis studied over a period of years. The author has made a composite picture of all the clinical phenomena observed in this group, which he calls rheumatic disease. *Streptococcus haemolyticus* was found to be the predominant organism in the upper respiratory tract at the onset of rheumatic attacks in almost all patients. The author observed that hemolytic streptococci are seldom found in the throats of people living in Porto Rico, and that rheumatic fever rarely occurs in the tropics. Ten patients with persistently active rheumatic disease were sent to Porto Rico for six months. They showed considerable improvement, and the hemolytic streptococci disappeared from their throats. As soon as the patients returned to the United States, the same organisms reappeared in their throats and the rheumatic attacks began again. Tests of the skin with the nucleoprotein of *Streptococcus haemolyticus* showed decided reactions in almost all patients with active rheumatic disease. Case histories, most of which are repetitious, occupy a large portion of the book. A concise article would cover the material of importance.

DIE HISTOGENESE EKTO-MESO-DERMALER MISCHGESCHWÜLSTE. EIN BEITRAG ZUR FRAGE DER ORGANISATORENWIRKUNG (SPEMANN) BEIM PATHOLOGISCHEN WACHSTUM. By PROF. DR. PAUL SCHÜRMANN, DR. MED. HANS PFLÜGER and ZAHNARZT DR. WILHELM NORRENBROCK. Price, 11.50 marks. Pp. 94, with 79 illustrations. Leipzig: Georg Thieme, 1931.

This attractive monograph supports the thesis that odontomas of the hypophyseal duct and mixed salivary tumors are primary epithelial tumors, the part taken in their growth by mesenchymal elements being secondary and determined by the organizing influence of the oral epithelium. The senior authors (Pflüger and Schürmann) describe in detail two instances of complicated odontomas of the hypophyseal duct and discuss their morphologic and genetic relations to other tumors of the duct. Norrenbrock considers the histogenesis of mixed tumors of the parotid glands. Both parts have many and excellent illustrations. While the tumors under consideration are mixed in the descriptive sense, they are interpreted by the authors as being essentially and genetically epithelial. The complexity of their structure is ascribed to the many potentialities of the oral ectoderm. The mesenchymal elements are held to develop under and in response to an organizing influence on the part of the epithelial cells, but just how this influence is brought into action is not explained. The morphologic descriptions are thorough and authoritative.

Books Received

DIE LEBENSVORGÄNGE IM NORMALEN KNORPEL UND SEINE WUCHERUNG BEI AKROMEGALIE. Von Professor Dr. J. Erdheim a.o. Professor an der Universität Wien. Price, 18 marks; bound, 19.60 marks. Pp. 160, with 31 illustrations. Berlin: Julius Springer, 1931.

LEPTOSPIROSIS IN THE ANDAMANS WITH AN APPENDIX ON THE PRESENT KNOWLEDGE OF LEPTOSPIRAL INFECTIONS. By Lieut.-Col. J. Taylor, D.S.O., M.D., D.P.H., I.M.S., and Amar Nath Goyle, M.B., Ph.D. (from the Pasteur Institute of Burma, Rangoon), Indian Medical Research Memoirs no. 20. Price, 6-0-0 rupees or 8 shillings. Pp. 190. Calcutta: Thacker, Spink & Company, 1931.

THE RENAL LESION IN BRIGHT'S DISEASE. By Thomas Addis, Professor of Medicine, Stanford University Medical School, San Francisco, and Jean Oliver, Professor of Pathology, The Long Island College of Medicine, Brooklyn; formerly Professor of Pathology, Stanford University, San Francisco. Price, $16. Pp. 650, with 181 illustrations (some in color), and large table. New York: Paul B. Hoeber, Inc., 1931.

WILLIAM STEWART HALSTED, SURGEON. By W. G. MacCullum. Introduction by Dr. W. H. Welch. Price, $2.75. Pp. 246. Baltimore: Johns Hopkins Press, 1931.

STREPTOCOCCIC BLOOD STREAM INFECTIONS. By GEORGE E. ROCKWELL, M.A., M.D., Associate Professor of Bacteriology, College of Medicine, University of Cincinnati; Member of Senior Medical Staff, Bethesda Hospital; Assistant Director of Bacteriological Service, General Hospital of Cincinnati. Price, $1.75. Pp. 73. New York: The Macmillan Company, 1931.

CLINICAL DIAGNOSIS BY LABORATORY METHODS. By James Campbell Todd, Ph.B., M.D., Late Professor of Clinical Pathology, University of Colorado, School of Medicine; and Arthur Hawley Sanford, A.M., M.D., Professor of Clinical Pathology, University of Minnesota (Mayo Foundation); Head of Section on Clinical Laboratories, Mayo Clinic. Seventh Edition. Price, cloth, $6 net. Pp. 765, with 347 illustrations, 29 in color. Philadelphia: W. B. Saunders Company, 1931.

NUTRITION AND DIET IN HEALTH AND DISEASE. By JAMES S. MCLESTER, M.D., Professor of Medicine at the University of Alabama, Birmingham, Ala. Second Edition. Price, cloth, $8.50 net. Pp. 891. Philadelphia: W. B. Saunders Company, 1931.

EINFÜHRUNG IN DIE MEDIZIN. Von Dr. Med. Henry E. Sigerist, Professor an der Universität Leipzig. Price, 12.50 marks; bound, 14.00 marks. Pp. 405. Leipzig: Georg Thieme, 1931.

RESISTANCE TO INFECTIOUS DISEASES. AN EXPOSITION OF THE BIOLOGICAL PHENOMENA UNDERLYING THE OCCURRENCE OF INFECTION AND THE RECOVERY OF THE ANIMAL BODY FROM INFECTIOUS DISEASE, WITH A CONSIDERATION OF THE PRINCIPLES UNDERLYING SPECIFIC DIAGNOSIS AND THERAPEUTIC MEASURES. By Hans Zinsser, M.D., Professor of Bacteriology and Immunity, Medical School, Harvard University; formerly Professor of Bacteriology at the College of Physicians and Surgeons, Columbia University, and Bacteriologist to the Presbyterian Hospital, New York; formerly Professor of Bacteriology and Immunity, Stanford University. Fourth Edition. Price, $7. Pp. 651. New York: The Macmillan Company, 1931.

CONTRIBUICAO AO ESTUDO DA BIOLOGIA DO STRONGYLOIDES STERCORALIS A' MARGEM DE UM CASO RARO, PROVAVELMENTE INEDITO, DE ESTRONGYLOIDOSE. By Heitor Praguer Froes. Pp. 123, with 21 figures. Bahia: 1930.